The effect of cold water immersion on recreationally active young adults and the recovery of elite rugby players after intense eccentric exercise

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Declaration

No portion of this work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institution of learning.

Dedication

To My Mother: Salma

Acknowledgment

I would like to express my sincere appreciation to Dr. Jamie McPhee, who was my supervisor throughout the journey of my PHD program for his wise counsel, encouragement and providing me with ideas, direction and advising me in the statistical analysis of my data as well as his generosity in supporting me through the period.

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Abstract

Background

Exercising at different levels of intensity is associated with an acute inflammatory response as a result of muscle damage, which consequently leads to delayed onset muscle soreness (DOMS). Cold-water immersion (CWI) has shown the potential to reverse exercise related muscle inflammation and enhance post-exercise recovery following sport activities. Several studies have investigated the effect of CWI on muscle recovery, however, their results are controversial. The purpose of the current study was to examine the physiological response of CWI on healthy participants and explore the physiological and psychological effect of CWI on athletes compared with controls.

Methods

Phase one observed the physiological response of 9 healthy active volunteers immersed in 12-13° for 15 minutes. Heart rate (HR), blood pressure (BP) and O_2 consumption were measured and monitored. Inflammatory biomarkers and muscle strength were observed prior to immersion, 30 minute and 24 hour following CWI.
Phase two used a randomized crossover trial to study the effectiveness of CWI [15 minutes of CWI at 12-13°C] compared to passive recovery [15 minutes sitting] post delayed onset muscle soreness in 8 elite male rugby players. Inflammatory biomarker, muscle strength, were measured prior to the intervention and 30 minutes, 24 and 48 hour post intervention. Muscle soreness [VAS and pain pressure threshold] was measured 20 minutes, 24 and 48 hours post intervention. Phase three explored the psychological effect of CWI using a focus group and self-administered questionnaire.

Result

In Phase one, CWI produced no significant changes in cardiovascular function, oxygen consumption, muscle strength and hormone concentration levels.

In Phase two, CWI reduced immediate quadriceps muscle soreness by (5 unit) compare to passive group by (15 unit) (P=0.006). No effect on strength or inflammatory cytokines compared to passive recovery.

In Phase three, athletes reported a perception of improved performance and reduction in pain when using CWI.

Conclusion

CWI has positive effects for the treatment of DOMS. Physiologically it reduces immediate muscle tenderness, but does not affect muscle strength. Psychologically athletes perceive an improvement in performance and reduction in pain.

List of Abbreviations

CK	Creatine kinase
CPK	Creatine Phosphokinase
DOMS	Delayed Onset Muscle Soreness
E-C	Excitation-Contraction
EMG	Electromyogram
ES	Electric Stimulation
LDH	Lactate Dehydrogenase
Mb	Myoglobin
Min	Minute
MRI	Magnetic Resonance Imaging
MVIC	Maximum voluntary isometric contraction
NSAIDs	Non steroid anti-inflammatory
PCr	Phosphocreatine
PGE2	Prostoglandin E2
Pi	Phosphate
PUS	Pulsed Ultrasound
SMVIC	Superimposed Electrical Stimulation
TT	Time Trail

TWI	Temperate Water Immersion
IL-1β	Interleukin- 1β
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor-α
IFN-γ	Interferon Gamma
α-MSH	Alpha Melanocyte Stimulating Hormone
IL-10	Interleukin-10
Il-5	Interleukin- 5
IL-9	Interleukine-9
IL-17	Interleukine-17
IL-13	Interleukine-13
IL-15	Interleukine-15
GM-CSF	Granulocyte-macrophage colony-stimulating factor
CCL20	Chemokine (C-C motif) ligand 20
TNF-b	Tumor necrosis factor-b
IL-12 (p70)	Interleukin IL-12 (p70)

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Chapter One. Introduction

1.1 Background

Athletes are required to perform to an extremely high-level of physical activity on a regular basis, which is structured through elite sport training (Corbett et al., 2012). Consequently, an athlete will ultimately experience varying degrees of muscular damage and inflammation, as well as a heightened perception of muscular soreness, which is generally labeled as “delayed onset muscle soreness (DOMS)” (Connolly et al., 2003). Moreover, another consequence in the subsequent days after exercise is one’s physical capability reduction (Ascensao et al., 2011).

Although DOMS is a generally experienced phenomena that can happen in any individual, independent of their fitness level, the exact mechanism(s) responsible for DOMS are not completely understood (Curtis et al., 2010). Thus, in an attempt to explain the underlying mechanisms that are responsible for DOMS, a number of theories have been proposed. The following theories have been included: lactic acid (Asmussen, 1956), muscle spasm (Devries, 1961), connective tissue and muscle damage (Hough, 1902; Abraham, 1976; Friden et al., 1983; Stauber, 1988), enzyme efflux (Armstrong, 1984), and inflammation (Smith, 1991).

To date, no research has been able to conclusively attribute any one mechanism as the primary cause of DOMS or its related sensations. However, much of the previously conducted research involved exercise-induced muscle damage, and events similar to those associated with an acute inflammation response have been documented. Therefore, various investigations have been structured to alleviate the soreness by formulating a distinctly effective recovery process. Optimal recovery between training bouts and competition is recommended to ensure that athletes can train frequently which will reduce the associated risks when treatment is not administered (Broatch et al., 2014).

An athlete's readiness to recommence training or competition must be the principle objective of any recovery session. Hence, a distinct period of physical and psychological regeneration has to be permitted through the post-exercise recovery. Physiological and psychological responses to recovery interventions are intimately linked and require adequate attention for the athlete (Stacey et al., 2010). As a matter of fact, a marked balance should be noted between the recovery of an athlete and the stresses endured by training, as the recovery period should combine both the alleviation of soreness and the restoration of performance.

One of the most utilised recovery interventions to advance the recovery time of an athlete post-exercise is cold-water immersion therapy, although at present this is established purely on anecdotal evidence (Barnett, 2006; Wilcock et al., 2006). In fact, it is not presently evident how the scientific rationale can be specifically applied to an athlete's recovery (Cochrane, 2004; Bleakley and Davison, 2009). Nevertheless, there are certain studies that have simultaneously demonstrated the physiological effects of post-exercise cold-water immersion, but with no precise evidence into the muscle strength recovery dynamics (Pendergast and Lundgren, 2009).

Different positive effects of cold-water immersion following exercise in contrast to passive rest have been established throughout various bodies of research, which have highlighted increased muscle power, as well as a direct reduction of muscle stiffness and soreness (Takahashi et al., 2006). In addition, researchers have ascertained an improvement in isometric strength and loaded squat jump performance following CWI compare to passive rest (Skurvydas et al., 2006; White et al., 2014). Simultaneously, a decrease in creatine kinase (CK) and perceived pain have been noted as additional positive effects of CWI compare to passive rest (Vaile et al., 2008). Moreover, improved muscle soreness and increased general fatigue have been shown by (Rowsell et al., 2009) following cold-water immersion when compared to passive rest.

Nonetheless, Because of methodological differences between studies athletes and coaches may become confused in regards to which intervention would be the most appropriate to implement (Glasgow et al., 2014). Overall, the physiological impact on cardiorespiratory, vascular and neuromuscular responses in damaged muscles has been shown to be effectual following cold-water therapy, even though data findings have remained unspecific (Yanagisawa et al., 2004;Wilcock et al., 2006; Bailey et al., 2007). Hence, the exact level of physiological responses following the therapy of CWI upon damaged muscles has yet to be established from findings and results of previous research and investigations, which the current study intends to clarify. Moreover, the neuromuscular reaction from undamaged muscles to the cold-water at rest is currently inconclusive, although the cardiorespiratory and vascular responses have been documented previously (Sramek et al., 2000).

Therefore, this study will be outlined through three distinct phases. Firstly, the physiological changes upon undamaged muscles during cold-water immersion will be analysed in healthy individuals who are not undertaking current physical activity. Subsequently, this will lead the second phase to investigate the impact of CWI on an elite group of physically active individuals, who consist of currently well-trained rugby players from the same team. Likewise, the third phase will specify the rugby players' perceptions of CWI post-training, and how the perceived improvement in muscle soreness, alongside physical performance improvement is effected by cold-water immersion. Additionally, the effective reduction of markers, from post-training muscle damage and inflammation by cold-water, will receive significant investigation throughout the first two phases.

1.2 Aims of the study:

- To describe the physiological response of healthy males when submerged in CWI (Chapter 5).
- To compare the effects of CWI on quadriceps muscle soreness and quadriceps muscle strength in two groups of athletes after training: those who receive the treatment and the control group who do not (Chapter 6).
- To explore the athletes' perceptions of taking part in CWI after training (Chapter 7).

1.3 Objectives of the study

1. To evaluate the effects of cold-water immersion on athlete recovery post-intense exercise through different measured post-exercise time-intervals.

2. To assess the effects of cold-water immersion on physiological recovery.

3. To assess whether there a relationship exists between physiological response, muscle function and perceived measures of recovery, as indicators of an athlete's overall recovery.

4. To evaluate whether the psychological response had an impact on the athletes' recovery.

5. To formulate innovative cold-water immersion practice recommendations based on the findings of this study.

6. To explore how evidence relating to the practice of cold-water immersion recovery is valued and duly applied by practice experts.

1.4 Hypothesis

1.4.1 Phase One

H_0: There will be no changes in the physiological responses and muscle strength to the cold-water immersion (12-13°C) for fifteen minutes in a group of healthy males.

H_1: There will be changes in the physiological responses and muscle strength to the cold-water immersion (12-13°C) for fifteen minutes in a group of healthy males.

1.4.2 Phase Two

H0: CWI will not decrease the level of soreness, or increase strength and inflammatory markers compared to the control group in rugby athletes following muscle damage.

H1: CWI will decrease the level of soreness, and increase strength and inflammatory markers compared to the control group in rugby athletes following muscle damage

1.5 Outline of the thesis

Having introduced the aim of the study and the research objectives, **Chapter Two** provides documented and perceived characteristic of muscle soreness in detail; in terms of the types of exercise that cause the soreness, signs and symptoms, as well as theories that explain the mechanisms of soreness and the differences and contrasts in recovery management.

Chapter Three describes the history of the cryotherapy, which includes the definition, as well as the different applied methods and mechanisms of cold-water immersion during recovery. Moreover, a literature review is undertaken to identify and clarify any specific gap in the existing literature regarding the physiological response of the body whilst immersed in the cold-water at rest. Likewise, the gap in literature regarding the effect of cold-water immersion on the soreness and on the muscle strength after a degree of muscle damage in athletes is also investigated.

Chapter Four discusses the methodology that demonstrates the design of the study. It also describes the preparations carried out prior to the data collection: participant recruitment process, the different procedures used to gather the data, and planned data analysis.

The design and application of this section of the investigation is subdivided into three phases and is structured through the following three chapters (chapters 5,6,7).

Chapter Five (Phase one) investigates the physiological changes that a healthy male experiences at rest during immersion in cold-water in terms of heart rate, blood pressure, oxygen consumption. The Chapter also determines whether there is an impact of cold-water on muscle force production and muscle contractile properties. In addition, the results of the data and the discussion of the findings are also provided.

Chapter Six (Phase two) documents a randomised controlled cross-over trial. It investigates the effects of a single bout of fifteen minutes cold-water immersion (12-13°C) on muscle soreness, force production, muscle contractile properties and maximal voluntary isometric contraction muscle of the knee extensors (n=8), against the effects of seated passive recovery. This investigation followed a twenty minutes step-up exercise with the athletes wearing a 20 Kg vest weight. Additionally, an analysis of the data and discussion are also included.

Chapter Seven explores the effect of cold-water on body hormones such as muscle damage indicators and inflammatory markers, and also compares the level of these hormones during the time measurement period. Moreover, methods of blood analysis and their results are presented in this chapter.

Chapter Eight (Phase three) develops the research further and uses a mixed methods approach to investigate the perception of athletes regarding taking part in CWI after training, and to understand the rationale behind the usage.

Chapter Nine presents and discusses the findings from the three phases of the study and presents a general conclusion of the study, which leads to future recommendations, as well as the current limitations of the research.

Chapter Two. Delayed Onset Muscle Soreness

2.1 Background

In exercise in which the eccentric component is substantial, repeated and unaccustomed, the activity leads to damage in the exercise muscle fibers (Bowers et al., 2004). Loss of eccentric muscle strength becomes directly apparent immediately following the muscle activity through knee extension, although the pain, tenderness, swelling and stiffness develop more slowly and are most prominent following the muscle activity and especially throughout the initial days after the causative exercise. This is precisely the reason that this constellation of symptoms is often called Delayed Onset Muscle Soreness (DOMS) (Allen, 2001), which is a sensation of discomfort felt in skeletal muscle that is associated with movement or palpation, 24 to 72 hours following unaccustomed muscular exertion (Vickers et al., 1997; Lieber and Fridén, 2002). Nevertheless, it must be noted that no perception of soreness is usually experienced by the person in discomfort when they are in a stationary state of rest (Smith, 1992).

The sensation of discomfort following overuse of a muscle can be severe and appears to characterize as pain and soreness (Connolly et al., 2003). This localisation of pain can be attributed to a high concentration of muscle pain receptors in the connective tissue of the myotendinous region (Sethi, 2012). The myotendinous junction is characterised by a membrane that is continuous, extensively folded and interdigitated with the muscle cells. Additionally, the oblique arrangement of the muscle fibres leading to the myotendinous junction reduces the ability to withstand high tensile forces (Allen, 2001). As a result, the contractile elements of the muscle fibres in the myotendinous junction are vulnerable to microscopic damage (Cheung et al., 2003).

DOMS is frequently seen in novice athletes and also in athletes who have dropped out of physical activities, or have not trained for a period of 6 to 8 weeks – by which time the muscle has lost the protective adaptation effects of training – or athletes who modify their training regime to incorporate new and therefore unfamiliar components to their exercise routine (Selkar et al., 2010). Moreover, DOMS may be experienced after performing eccentric exercise. It has also been reported that exercises that mainly consist of large amounts of lengthening (eccentric) contractions produce greater DOMS than exercise that involves mainly shortening (concentric) contractions (Tiidus, 2008).

The practical consequences of DOMS include muscle soreness and tenderness, temporary reduction of force production, loss of strength (Scott et al., 2003), pain, stiffness, swelling and elevated levels of the enzyme creatine kinase, all of which do affect overall performance (Rocha et al., 2012). In fact, symptoms can vary from mild muscle tenderness to severe deliberating pain (Cheung et al., 2003).

Subsequently, DOMS has been a well-researched phenomenon and the morphological injury to the muscle has been previously well described (Proske and Morgan, 2001). The first report on DOMS was published in 1902 by Theodore Hough, who suggests that soreness was most likely due to some sort of rupture within the muscle (O'Connor and Hurley, 2003). Since then, a considerable volume of research has emerged and a number of theories regarding the patho-physiological mechanisms of DOMS have been proposed (Curtis et al., 2010).

2.2 Eccentric Contractions

Although DOMS may occur following unaccustomed effort, the most predictable way of inducing it is to expose the muscle to high strength repeated eccentric contractions in which the activated muscle is pushed to elongate while active tension is produced (Asmussen, 1956; Friden et al., 1986; Newham, 1988; Lieber and Fridén, 2002). Thus, eccentric exercise is defined as a type of contraction where the muscle contraction is lengthening while tension is developed (Jamurtas et al., 2005). In athletic competition, eccentric muscle force is required for activities such as cycling, throwing, hiking downhill, landing, and decelerating after running (Enoka, 1996). Nonetheless, eccentric contractions also frequently occur in everyday activities such as descending stairs, squatting and lowering objects (Proske and Morgan, 2001). As a consequence of these activities, eccentric contractions subject the muscle to higher tensions than concentric contractions at a given level of submaximal speed, as the concentric contractions are much shorter throughout smaller range of motion (Semmler et al., 2007). In addition, there is significant evidence that eccentric contractions cause more damage than other types of contractions (Hamlin and Quigley, 2001; Kazunori et al., 2002).

2.3 Clinical Signs and symptoms

Pain during movement, decreases range of motion in the joint (Dutto and Braun, 2004), muscle tension, decreased muscle strength, increased limb volume (oedema) and the presence of muscle enzymes, such as Creatine Kinase and lactate in blood, are the common symptoms of DOMS (Eston et al., 2003; Martinez-Amat et al., 2005). However, there is no structured evidence of any long-term damage or permanent reduced muscle function being caused by DOMS (Armstrong, 1984). Hence, measuring the clinical signs of swelling, range of movement, and strength, as well as the evaluation of pain, is the most appropriate way to monitor and manage the effects of DOMS (Cheung et al., 2003).

2.3.1 Pain

Soreness in the area of the musculotendinous junction typically appears between 4 to 8 hours post-eccentric exercise, peaks at 24-48 hours and can last for up to 7 days (Curtis et al., 2010). Moreover, DOMS may leave individuals in a state of moderate to severe pain, depending on the intensity of the activity and their fitness levels (Soer et al., 2009).

Muscle soreness needs to be differentiated between temporary soreness and DOMS, as temporary soreness is usually felt during the final stages of fatiguing exercise and is believed to be caused by metabolic waste (Croisier et al., 2003), whereas DOMS is usually felt during movement or on palpation of the affected muscle at least 8 hours post exercise (Lorenz and Reiman, 2011).

The exact mechanism responsible for the delay of pain is not fully understood. However, it has been proposed (see Figure 1) that eccentric exercise may cause injury to the cell membrane, which will then set off an inflammatory response that consequently leads to prostaglandin (prostaglandin E2 [PGE2]) and leukotriene synthesis (Connolly et al., 2003). Subsequently, the overall elevation in these inflammatory substances has been implicated in producing a heightened sensation of soreness (Rossato et al., 2015). Prostaglandin E2 directly induces the sensation of pain by sensitizing type III and IV pain afferents to the effects of chemical stimuli, whereas leukotrienes raise vascular permeability and initiate neutrophils to the point of damage (Zainuddin et al., 2005).

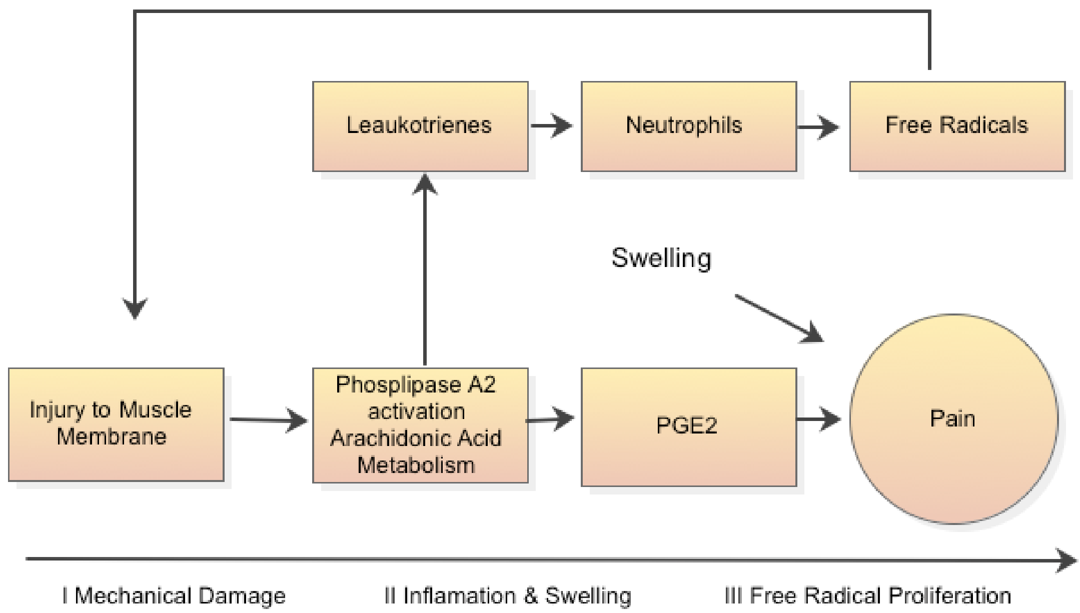


Figure 2.1 Schematic showing possible sequence of injury (Connolly et al., 2003)

2.3.2 Swelling

Swelling is an inflammatory response of the damaged muscle fibre, as the damage causes a transfer of fluid and cells to the affected muscle (Eston et al., 2003). Subsequently, the attraction of fluid frees proteins which have accumulated in the interstitum following injury, and this accumulation results in a disruption of normal capillary filtration pressure, allowing for swelling to occur (Denegar, 2000). Exercise results in intramuscular oedema that activates mechanoreceptors and subsequently causes pain, which is triggered by swelling (MacIntyre et al., 1995), as well as the increase in local tissue pressure (Connolly et al., 2003).

2.3.3 Range of motion reduction

Human movement is dependent on range of motion (ROM) available in synovial joints, which may be limited through injury to two anatomical structures: muscles and joints (Page, 2012). Hence, decrement in the range of movement will ultimately affect performance (O'Connor, 2003).

Decrement in range of motion tends to start reducing with DOMS at 48hrs after an intense eccentric bout of exercise (Dutto and Braun, 2004). Moreover, it seems that swelling in preimuscular connective tissue, especially in the regions of myotendinal junctions, can restrict movement as well as decrease levels of strength (Lee et al., 2002).

2.3.4 Strength Reduction

Strength loss is most notable after eccentric exercise and is considered the most accurate and reliable indirect measure of muscle damage (Cheung et al., 2003). Within 2 hours of the muscle-damaging bout of exercise a loss of force that can generate force or strength has commonly been reported to have become compromised (MacIntyre et al., 1995). In addition, it is believed that the mass of muscle required to produce force is decreased as a result of damaged contractile units (Newham et al., 1991). However, it has not been proven whether force deficits are entirely due to myofilament damage or whether they are likewise attributable to variations in excitation-contraction (E-C) coupling (Behm et al., 2001). Besides the actual cause, the duration of strength reduction before returning to a normal baseline after eccentric exercise may be 8 to 10 days (Ebbeling and Clarkson, 1989).

2.4 Negative Impact of DOMS on athletic performance

In general, athletic performance and preparation are typically impaired when an athlete experiences soreness or injury, as due to the damage to the muscle and connective tissue during eccentric activity, muscle function and joint mechanics can be affected and can cause a significant reduction in any individual performance (Nguyen et al., 2009). Rowlands et al. (2001) report that performing exercise with tender and weaker muscles could impair performance, as implemented force becomes compromised. Furthermore, numerous researchers have documented that reductions in range of motion, power and strength can also result in a decline in muscle performance such as counter movement jump and sprint distance (Smith, 1992; Eston et al., 2003).

Overall, strength is an important measure of athletic performance, as research has generally demonstrated a significant reduction in strength/power parameters, associated with DOMS. Individuals who suffer from DOMS as a result of unaccustomed exercise have shown a significant reduction in eccentric force production (Close et al., 2005). In fact, reduced maximal force is potentially secondary to both sarcomere 'popping' and disorganization (Morgan, 1990), together with component damage of the coupling process between excitation and contraction (Warren et al. 1993; Balnave et al. 1997).

The potential loss of eccentric and concentric strength may have implications for athletic performance, although, the reductions are more profound and more persistent during eccentric testing (Eston et al., 2003). Furthermore, peak torque deficits are most pronounced immediately after exercise, with little restoration at 24 and 48 hours (Allen, 2001). Recovery may be slow, and may require 8 to 10 days to return back to a normal baseline level (Cleak and Eston, 1992; Howell et al., 1993; Cheung et al., 2003). As a matter of fact, muscle strength does not return to its baseline as quickly as pain and range of movement recover following eccentric exercise (O'Connor and Hurley, 2003). At high frequency stimulation, muscle force is reduced by approximately 30% in the eccentrically contracted muscle and recovers over 24 hours, whereas the reduction of force in the concentric contracting muscle is only approximately 8% and only apparent for a few hours (Allen, 2001). However, force can remain depressed for up to a week after eccentric contractions (Proske and Allen, 2005). Ebbeling and Clarkson, (1989) suggest that there is very little, or no, relationship between the development of soreness and a decrease in muscle strength. Accordingly, muscle fibre damage does not necessarily cause pain, as the pain within muscles results from secondary events that occur following the damage itself (Cleak and Eston, 1992).

Another factor that can lead to a decrease in muscle strength due to soreness may be attributable to structural or organisational damage of the sarcomeres, such as the tearing of the z-lines (Nguyen et al., 2009). An explanation for the loss of strength after eccentric exercise (Clarkson et al., 1992) suggests that the sarcomeres are stretched under tension , particularly those which are shorter towards the end of the muscle fibres. This reduces the overlap between the actin and myosin filaments and decreases the number of cross bridges that could potentially form.

The duration of the strength loss after muscle damaging exercise remains equivocal, as muscle strength deficit may take up to two weeks for maximal recovery depending on the severity of the initial damage and on the muscle group(s) affected (Cleak and Eston, 1992;

Falvo and Bloomer, 2006; Mancinelli et al., 2006). A review by Eston et al., (2003) indicated that relatively inactive elbow flexors tend to show a heightened risk of persistent functional deficit, as opposed to the behaviours of locomotor muscles in the lower limbs. Differences within the architecture of upper and lower muscle extremities produce contrasting mechanical stress per muscle units when performing eccentric exercises of the same intensity (Saka et al., 2009), possibly explaining the difference in duration of recovery between the upper and lower limbs. It has been stated by Jamurtas et al. (2005) that during daily activities, submaximal activities that have an eccentric component, such as walking downhill or downstairs, is a routine training stimulus for knee extensors and plantar flexors, while the elbow flexors experience fewer intense eccentric contractions during normal daily activities. After a bout of eccentric exercise the muscles adapt and are subsequently less susceptible to further damage following repeated bouts of eccentric exercise (Clarkson and Hubal, 2002; Proske and Allen, 2005). Therefore, this could be the reason for the different muscle damage responses between the leg and arm muscles.

Following exercise-induced muscle damage, the knee and ankle extensors demonstrate that immediate and prolonged isometric strength reduction and recovery were faster (4-7 days) than observed for the elbow flexors.

2.5 Creatine Kinase level increase

Creatine Kinase (CK) is an intramuscular enzyme responsible for maintaining adequate adenosine triphosphate levels during muscle contraction (Lieber and Fridén, 2002). Creatine Kinase is found in muscle tissue and is released into the circulation when muscle damage occurs. Therefore, in exercise physiology, serum CK has been used as an indirect marker in DOMS for the detection of muscle injury (Kaznori et al., 2002; Naiya, 2012). In fact, research has shown that after being subjected to eccentric and sustained exercise, female subjects tend to have lower levels of Creatine Kinase activity in comparison to their male counter-parts, thus, female subjects sustain less muscular damage as a result (Clarkson and Hubal, 2001).

2.6 Theories regarding the Mechanisms of DOMS?

There are many theories that describe the reason for DOMS. Subsequently, when one attempts to prevent and alleviate DOMS it is important to have an understanding of the underlying mechanisms that contribute to DOMS. These mechanisms include damage to the contractile elements of muscles and associated connective tissue (Hough, 1902). In addition, the inflammatory process associated with micro-injury, to both the contractile and non-contractile properties of muscle, needs to be comprehended (Smith, 1991). DOMS is assumed to be initiated by the mechanical disruption of muscle fibres at a cellular level, and a reduction in maximal strength followed by an increase in the inflammatory markers level.

2.6.1 Lactic Acid Theory

Lactic acid is a natural product that the body releases as a result of eccentric exercise. It is secreted into the muscle tissue and causes irritation, which consequently causes sensations of muscle soreness. As a result, these feelings of discomfort have been attributed to symptoms of fatigue (Braun and Dutto, 2003)

The lactic acid theory is based on the assumption that lactic acid continues to be produced following exercise cessation. However, on reviewing the literature it appears that this is unlikely to be the case, due to different reasons (Newham et al., 1983). Firstly, following a stepping exercise, the delayed onset of pain is experienced in the leg that has done the least metabolic work (Szymanski, 2001). Eccentric exercise entails minimal metabolic cost and it is unlikely that there would be a significant accumulation of lactate (Szymanski, 2001). Secondly, the pain is apparent 24-48 hours after exercise and lactic acid levels return to pre-exercise levels within 1 hour of post-exercise (Jones et al., 2004). Therefore, whilst lactic acid can contribute to the acute pain associated with fatigue following intense exercise it cannot be attributed to the delayed pain that is experienced 24-48 hours post-exercise (Cheung et al., 2003). Due to the aforementioned reasons, it is unlikely that lactic acid accumulation can be the underlying mechanism which causes DOMS.

2.6.2 Spasm Theory

In 1961, DeVries described an original concept on muscle spasm theory. The theory suggests that an increase in resting muscle activation indicates a tonic localized spasm of motor units, which leads to ischaemia. As a result, an ischaemic muscle results in the production of a pain substance called ‘P’. Thus, once sufficient quantities of the substance have accumulated, ‘P’ stimulates pain nerve endings within the muscle and in turn the pain produces further reflex spasms and prolonged ischaemic conditions (Francis, 1983). Nonetheless, electromyogram (EMG) studies have failed to demonstrate an increased activity in sore muscles or a correlation of activity to soreness following exercise.

Therefore, the muscle spasm theory has been largely discounted (Abraham, 1976; Howell et al., 1985; Bobbert et al., 1986).

2.6.3 The Muscle Damage or Torn Connective Tissue Theory

There are two theories on muscle damage and torn connective tissue. Both theories are consistent, particularly with the timing of soreness that occurs following eccentric loads. The muscle damage theory, first proposed by Hough, (1902), focuses on the disruption of the contractile component of muscle tissue, particularly at the level of the z-line, following unaccustomed exercise. When the sarcomeres are pulled apart, there would be a subsequent reduction in overlapping actin and myosin filaments, which ultimately reduce muscle peak force (Morgan, 1990). Likewise, during eccentric action a reduction of force in the active motor unit causes increased tension per unit area, which leads to a disruption of the contractile component. As a result of the disruption, nociceptors that are situated in the muscle connective tissue and in the region of the arterioles, capillaries, and the musculotendinous junction will release CK. Also, the stimulation of pain receptors leads to undesirable soreness or the sensation of pain. Armstrong, (1984), actually states that increased tension per unit area could cause the mechanical disruption of structural elements in the muscle fibre or in the connective tissue that is in series with contractile elements. There are also indications that connective tissue breakdown is part of the underlying mechanism that causes DOMS.

The torn connective tissue damage theory also investigates the role of the connective tissue that forms sheaths around bundles of muscle fibres, which have been supported by post-exercise measurements taken from urine amino acids excretion of hydroxyproline (HP) and hydroylysine (HL) (Sydney-Smith and Quigley, 1992). Indeed, the presence of it in the urine is due to collagen degredation, which is caused by damage from strain or overuse (Stauber, 1989). Connective tissue surrounds the muscle fibres in varying degrees, with type I fibres (slow twitch) displaying a more robust structure than type II fibres (fast twitch). Therefore, stress placed on the muscle will have a greater injurious effect on type II fibres due to the connective tissue's inability to withstand tension at the same level as type I fibres. Simultaneously, the excessive strain on the connective tissue may also lead to increased muscle soreness (Proske and Morgan, 2001).

Furthermore, the torn connective tissue theory is supported by studies that have used myoglobin as trace markers of muscle damage to describe the disruption of subcellular components. As a consequence of these disruptions, damage is caused to the sarcoplasmic reticulum and T tubule; both of which interfere with normal calcium metabolism (Francis, 1983).

2.6.4 Inflammation Theory

On finding aspects of inflammatory responses, the inflammation theory shows evidence, which suggests that following repetitive eccentric muscle action, inflammatory responses play a role in the degeneration and regeneration of damaged muscle (Cheung et al., 2003).

Smith (1991) proposes that acute inflammation is the mechanism that underlies DOMS. According to this theory, neutrophils are released into the circulation due to tissue disruption after eccentric exercises and migrate to the damaged muscle tissue within several hours. At the site of injury, neutrophils release chemicals, which are dispersed into injured cells. Following this, the second influx of white blood cells; known as macrophages, begin to infiltrate 8-12 hours following exercise, directly into the damaged areas. It is at this stage where large amounts of prostaglandin (PGE2s) are synthesized along with the injured cells. Yet, despite the fact that PGE2s are not directly the cause of pain, they do stimulate sensitivity in the pain receptors, which leads to the sensation of DOMS. Subsequently, PGE2s are believed to be significant mediators of inflammation (Prisk and Huard, 2003).

Contrastingly, at present, many experts (Mair et al., 1995; Chen and Hsieh, 2000; Nosaka et al., 2002) all agree that some aspects of the inflammatory response are seen secondary to either muscular or the connective tissue damage following strenuous eccentric activity.

2.6.5 Enzyme Efflux Theory

The enzyme efflux theory is based on a model developed by Armstrong (1984), in which calcium from the interstitium accumulates in injured muscles following sarcolemma damage. High mechanical forces produced during eccentric muscle activity cause disruption of structural proteins in muscle fibres, particularly at the weak z-lines. This is accompanied by excessive strain on the connective tissue between the active cross bridges. Furthermore, inducing injury to the sarcolemma can potentially incur progressive, indirect compromise to its own structure, thereby enabling the activation of proteases and phospholipases, which is followed by the production of leukotrienes and prostoglandins. Moreover, the interstitium and plasma can also be infused with intracellular components.

These components in turn attract monocytes into the localised injury and can manifest themselves into macrophages, which activates mast cells and histocytes into the specific point of injury. As a result, these processes become the progressive deterioration of the sarcolemma's structure and are all believed to be a direct result of prior eccentric exercise (Armstrong et al., 1991). Furthermore, additional consideration is taken when emphasis is applied on the stimulus of group IV sensory neurons' free nerve endings, which is a result of a build-up of histamine, potassium and kinins. Therefore, nocioceptors are activated, that causes a sensation, namely DOMS.

2.6.6 Theory Sequence

According to the majority of researchers (Smith, 1991; Cheung et al., 2003; Gulick and Kimura, 2010), a single theory cannot explain the onset of DOMS, as it may result from a sequence of events involving the rupture of connective tissue, muscle damage, enzyme efflux, and inflammation. Hence, a hypothetical sequence of series has been suggested in order to attempt an explanation regarding the mechanisms causing DOMS (Armstrong, 1984; Smith, 1991).

Following a review by Armstrong (1984), Smith’s sequence (1991) starts with an assumption that during eccentric muscle activity a high tensile force causes the disruption of structural proteins in muscle fibres (connective tissue and muscle damage theory). As a result of the sarcolemma damage, calcium accumulates and inhibits cellular respiration (enzyme efflux theory). Intracellular components and markers of connective tissue and muscle damage diffuse into the plasma and interstitium. Mast cells and histamine production are activated and neutrophils will concentrate at the injury site within a few hours (inflammation theory). Moreover, upon exposure to this inflammatory environment, macrophages produce PGE2 that sensitizes type III and IV nerve endings to mechanical, chemical, and/or thermal inflammation (inflammation theory). The accumulation of histamine, potassium, and kinins from active phagocytosis and cellular necrosis, in addition to elevated pressure from tissue oedema and increased local temperature, then activate nocioceptors leading to the sensation of DOMS (inflammation) (Cheung et al., 2003).

The flow chart below proposes a sequence for the development of DOMS following unaccustomed eccentric exercise (Figure 2).

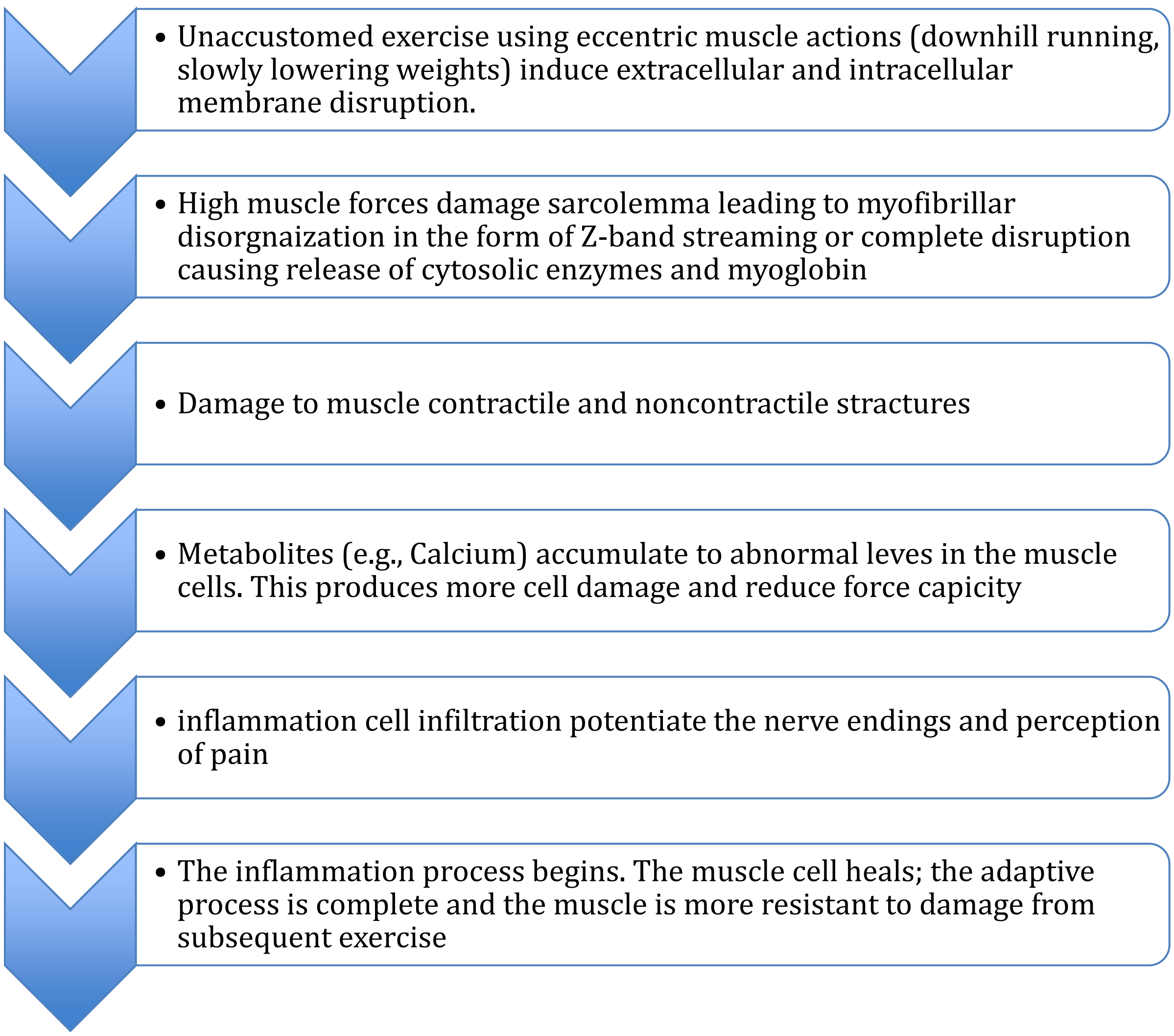


Figure 2.2 Sequence for the development of DOMS following unaccustomed eccentric exercise (Yu, 2003)

2.7 Measures of Muscle Damage

Muscle damage can be measured directly or indirectly. Specifically, direct assessment is possible through the analysis of muscle biopsies or, in the case of more severe damage, through magnetic resonance imaging (MRI). Muscle tissue tears are visible in MRI or, indirectly, increased muscle volume is interpreted as oedema occurring as a consequence of damage, although the direct link between eccentrically-induced damage and structural changes evident in MRI has not been demonstrated (Fulford et al., 2015; Malm et al., 2000).

Biopsies are taken to examine possible muscle damage, although taking a biopsy involves extracting a small tissue sample, which in itself can create two possible dilemmas (Clarkson and Hubal, 2002). Firstly, the act of extracting only small samples of tissue can mean that the test result may only reveal specific localised muscle damage, as opposed to assessing the area itself and the surrounding areas in general (Baird et al., 2012). Secondly, the act of extracting tissue samples can itself directly lead to further damage as a result of the invasive technique (Totsuka et al., 2002).

As assessment techniques, muscle biopsies and MRIs offer some inclination to possible tissue damage, but both methods cannot precisely quantify the full extent of muscle damage. As a consequence, many of the indicative findings can lead to diagnosis that is influenced by the subjective interpretation of the affected individual (e.g. athlete) and professional making the diagnosis (e.g. physiotherapist), which potentially affects the overall prospective care and recovery regime (Clarkson and Hubal, 2002).

Commonly used indirect markers of muscle damage after strenuous exercise include the measurement of different muscle proteins, such as creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (Mb), which are released into the circulation from damaged muscle tissue (Clarkson et al., 2006; Bailey et al., 2007). Of these, CK has used most frequently, but Mb is thought to allow earlier detection of muscle injury than CK (Sorichter et al., 1998). The time of CK release into the tissue and clearance from plasma depends on the level of training, type of activity, intensity and duration of exercise (Brancaccio et al., 2007). The highest post-exercise serum enzyme activity is found after very prolonged competitive exercise, such as marathon running and weight-bearing exercises, which include eccentric muscular contractions, as with downhill running (Brancaccio et al., 2010). CK has a delayed appearance in the blood, which means that it does not begin to increase until around 24–48 hrs following the damaging activity and peaks 96–120 hrs post-exercise (Clarkson et al., 1992). Myoglobin, however, is a much smaller protein than CK, which appears in the blood earlier (0.5-1hr post) and peaks sooner (24–72 hrs post) than CK (Nosaka and Clarkson, 1996). Meanwhile, LDH increases by the third and the fifth day after exercise (Brancaccio et al., 2008).

2.8 Treatment of DOMS

DOMS is a major complication that is faced by athletes after eccentric activities, which can compel them to postpone their sporting activity (Bakhtiary et al., 2007), necessitating the search for effective treatment modalities and protocols, in order to minimise the effect of DOMS and prevent or reduce the severity of its clinical signs (Connolly et al., 2003). Intervention is aimed towards limiting the extent of muscle soreness and one possible intervention is CWI. In turn, by adhering to an implemented pain reduction focused care plan, an affected person or athlete may reduce the duration of soreness. The expectation is that recovery is accelerated and the person can return to usual activities sooner (Anbarian et al., 2011) due to restoration of the neuromuscular function, in particular the ability to perform eccentric contractions without any inhibition or other form of weakness resulting from damage (Gulick et al., 1996). Moreover, by implementing a clear useful treatment plan prior to any muscle damaging event, a clinician can become more economic with time and cost management (O'Connor and Hurley, 2003; Weerapong and Kolt, 2005).

The strategies proposed to alleviate DOMS can be classified into two categories:

(a) Therapeutic treatments, which include pre and post exercise stretching, therapeutic ultrasound, electrical stimulation, massage and cryotherapy (cold-water immersion)
(b) Pharmacological treatment through the use of non-steroidal anti-inflammatories (NSAIDS) (Connolly et al., 2003).

Hilbert et al. (2003) states that none of these treatments attenuates DOMS. Alternatively, other investigators have found that some of these modalities, as well as cryotherapy, are useful in more than one domain (Connolly et al., 2003). More specifically, cold-water immersion is one of the most utilised methods of treatment to alleviate the symptoms of

DOMS (Bailey et al., 2007; Hausswirth et al., 2011).

2.9 Therapeutic Treatments

2.9.1 Stretching

Stretching is the application of force to musculotendinous structures in order to achieve a change in their length, usually for the purposes of improving joint range of motion, reducing stiffness or soreness, or preparing for activity (Armiger and Martin, 2010).

It was proposed that the eccentric exercise leading to DOMS might be initiated by a disruption of some of the intermediate filaments (e.g. desmin, titin) (Allen, 2001). Titin is likely involved as a "protein ruler" to regulate the assembly of myosin and actin precisely, and as the elastic component when a passive muscle is stretched (Tskhovrebova and Trinick, 2010). It is located between the M-line and the z-band. If a resting muscle is stretched passively it would thus affect the z-bands, by analogy to that which is seen during eccentric exercise. It could thus be argued that both stretching and eccentric exercise leading to DOMS affect the muscle tissue in almost the same way (Lund et al., 1998)

The effects of pre- and post-eccentric exercises stretching have been researched, and the effect upon DOMS has been shown to be limited although positive (Johansson et al., 1999; Herbert and Gabriel, 2002; Anbarian et al., 2011)

The effect on the occurrence of EIMD upon the hamstring of one leg prior to eccentric training was measured following 4x20 second stretching exercises (Johansson et al., 1999). Subsequently, there was no marked alteration between the control leg and the stretched leg during the warm-up. Likewise, there was no improvement to the outcome of DOMS following post-exercise stretching (Wessel & Wan, 1994).

Separately, a group of athletes that performed static stretching was studied following 30 minutes of training that focused on eccentric quadriceps and triceps exercises (Buroker et al., 1989). As a result, it was noted that in comparison to the control group, there was no reduction in strength in the 3 days post-training within the focus group.

Other researchers suggest that a combination of stretching and massage actually produces a significant reduction in the muscle soreness (Rodenburg et al., 1994; O'Connor and Hurley, 2003; Weerapong and Kolt, 2005). However, no significant beneficial effects of only stretching, in relation to DOMS treatment.

2.9.2 Ultrasound

Pulsed ultrasound (PUS) is an electrotherapeutic modality that has typically been used to decrease the symptoms of inflammation (pain and oedema) and to increase the rate of healing post DOMS (Hasson et al., 1989). According to Stay et al. (1998), using PUS post DOMS because of the mechanical effects, stable cavitations and microstreaming are believed to aid tissue regeneration and healing. Furthermore, changes in calcium permeability are associated with enhancing tissue healing and an increase in sodium permeability may reduce pain (Kachanathu et al., 2013).

Several studies have found that PUS does not diminish the effects of DOMS on soreness perception (Craig et al., 1999; Gulick et al., 1996; Aytar et al., 2008). Therefore, little conclusive evidence has been found to support the positive effect of ultrasound therapy in the management of DOMS (Craig et al., 1999; Tiidus et al., 2002; Aytar et al., 2008).

2.9.3 Electrical Stimulation

Electrical stimulation (ES) uses an electrical current to cause a single muscle or a group of muscles to contract, and it has been shown that ES is effective in pain relief. This is achieved by blocking the transmission of the pain impulse and hastening the healing process, as the sensory nerve is stimulated (Garrison and Foreman, 1994). However, it shows that, given the correct dose of mechanical stimulus, the increased presence of inflammatory cells in human skeletal muscle is a normal physiological response, where damage and remodelling have occured (Mackey et al., 2008).

A study conducted by (Allen et al., 1999) utilised a doubled blind research design requiring 18 participants (3 men and 15 women) to perform concentric and eccentric elbow flexion of non-dominant arm. At task failure, defined as an inability to appropriately control the movement, participants were assigned to either receive treatment with microcurrent stimulation at time intervals: 10 minute, 24, 48, 72 hours after DOMS, or sham group treated using a disabled machine. The microcurrent treatment was not effective in reducing pain associated with DOMS. A study conducted by (Crameri et al., 2007) utilised various untrained men who separately performed 210 maximal eccentric contractions on both legs. One leg was by voluntary contractions, and the other through electrically induced (ES) contractions. The study concludes that damage to the z-lines and myofibre proteins were only present in the ES group. As a result, one can see that the greater activation of fast muscle fibres at a given force level in ES is responsible for the greater muscle damage (Nosaka et al., 2011). Furthermore, it raises the question to how excessive the actual damage can ultimately be when ES force is applied. For instance, the study by Crameri et al. (2007) showed that damage to the z-lines and myofibre proteins were only present in the ES group, which experienced a 13% loss of MVC. Besides, no effective evidence exists at present, which can recommend the use of electrical stimulation to reduce the signs of DOMS (Butterfield et al., 1997; Allen et al., 1999; Denegar, 2000; Leeder et al., 2011).

2.9.4 Massage

Massage is widely used as a therapeutic modality for recovery from muscle injury. It has been proposed that during the early stages of inflammation, if massage is applied, the neutrophil margination becomes reduced due to mechanical pressure, which simultaneously decreases DOMS (Ernst, 1998). Ernst carried out a systematic review and concluded that even though massage has some potential in reducing the symptoms of DOMS, its effectiveness has not been demonstrated convincingly. However, a study by Hilbert et al. (2003), which included 18 volunteers randomly assigned to a 20-minute massage or a control group, determined that a massage has the inability to change circulating nutrophil levels, and as a consequence muscle damage caused by inflammation would not be attenuated. This was conducted after the volunteers had all performed six sets of eight maximal eccentric contractions. In fact, an 8-minute massage immediately post-exercise has no effect on DOMS and the recovery of muscle strength (Weber et al., 1994). Moreover, other researchers have reported that massages actually have no effect in relieving symptoms related to DOMS (Hilbert et al., 2003; Zainuddin et al., 2005; Mancinelli et al., 2006).

2.9.5 Cryotherapy (Cold-water immersion)

Cryotherapy is a term that describes a range of therapeutic treatments and can be applied either in the form of ice packs, an ice massage or cold-water immersion (CWI) (Meeusen and Lievens, 1986). The focus of this study, though, will mostly be based around cold-water immersion as it is the least expensive, most accessible and popular treatment for skeletal muscle injuries (Bleakley et al., 2004; Oliveira et al., 2006).

The therapeutic mechanisms of CWI are to induce vasoconstriction and may also reduce muscle blood flow, which bring about a reduction in muscle temperature, swelling and pain, and subsequently reduce post-exercise inflammation (Karunakara et al., 1999; Leeder et al., 2011). This proposed reduction in inflammation might be related to attenuating DOMS and enhancing the recovery of muscle function. Thus, an attempt to increase the rate of recovery may be beneficial to elite athletes (Leeder et al., 2011).

Several studies have used protocols that consist of eccentric actions to induce muscle damage in order to determine the efficacy of cryotherapy treatment. A study conducted by (Eston and Peters, 1999) shows that cold-water immersion is effective in providing some relief for DOMS. Stiffness and plasma CK activity decreases when cold-water immersion is applied for 15 minutes (min) immediately after exercise consisting of eccentric elbow flexors. Additionally, (Yanagisawa et al., 2003a; Yanagisawa et al., 2003b) also report some beneficial effects of cold-water immersion on exercise-induced muscle oedema and conclude that muscle soreness and CK activity become decreased as a result of the treatment, which will be discussed in-depth in Chapter Three that focuses on how CWI affects DOMS.

2.10 Pharmacological Treatment

One of the many treatment modalities advocated to facilitate the recovery of muscle function and alleviate the symptoms of DOMS is Non Steroid Anti-Inflammatory Drugs NSAIDs (Connolly et al., 2003). The easy accessibility of NSAIDs over the counter, and their reported ability to relieve pain and soreness resulting from participation in sport, has led to widespread use among athletes (Lanier, 2004).

Several authors report that the use of NSAIDs in response to exercise-induced muscle damage is not beneficial when administered before and after bouts of lower extremity eccentric exercise, and it neither alleviates soreness or the degree of inflammation associated with it (Donnelly et al., 1990; Gulick et al., 1996; Hertel, 1997). In fact, there have been concerns that the use of NSAIDs, in attempting to treat DOMS, may actually negatively interfere with the inflammation process and delay the healing process (Prentice, 2003).

Furthermore, since CK is one of the markers of muscle damage, NSAIDs have been shown to have no significant role in the CK response of injured muscles (Trappe et al., 2002; Nieman et al., 2006). However, CK in not the only significant marker in muscle injury, as (McAnulty et al., 2007) has found that in addition to CK, the use of NSAIDs does not diminish any oxidative stress markers after exercise.

In addition, there is disagreement among doctors regarding the safety of NSAIDs therapy in the prevention and/or treatment of muscle injury. This is due to the adverse effects of using NSAIDs for long period, such as an increased incidence of stomach ulcers, and liver damage, as well as the consequential opportunity for misuse and abuse (Cheung et al., 2003; Lanier, 2003).

2.11 Conclusion

The search for treating DOMS has been extensive, although somewhat inconclusive. Numerous investigators have attempted to identify treatment for DOMS, though, currently there is no treatment method recommended as the gold standard, despite the high level of incidence in elite athletes. Thus, a gap has been left in this field of research for further study and practical implementation.

During recent years, it has emerged that cold-water immersion therapy, has been found to decrease the signs and symptoms associated with DOMS, following eccentric based activity, when applied as a practical science. However, the current research in this field that still remains is, to some extent, descriptive in nature but not conclusive. Consequently, the research contributes little to the understanding of the potential mechanisms behind the impact of specific, practical recovery strategies. Therefore, continued research is still required, in order to fully comprehend the mechanisms underpinning cold-water immersion, which will be discussed further in the following section.

Chapter Three. The History and Application of Cold-Water Immersion

3.1 Background and Development

As early as 2500 BC, ancient Egyptians had applied the knowledge of the healing benefits of cold water and implemented these practices for the treatment of injury and inflammation that we still use in present day (Knight, 1995). The use of therapeutic cooling also dates back to the time of the ancient Romans and Greeks, where they mastered Cryotherapy. The term *cryo,* in fact, derives from the Greek *kryos*, meaning "cold", so Cryotherapy was an adaptation from cold-water therapy, although the only cooling modalities available in those times were ice and snow (Bradley., 2012).

The Greeks and Romans utilised snow, natural ice and cold-water therapy in order to ease the pain after acute trauma, or to reduce an inflammatory reaction (Wang et al., 2006). Moreover, around a hundred years ago a German scientist, Person Sebastian jumped into ice-cold water in an attempt to cure tuberculosis, which proved successful. This theorised that the disease can be overcome, even in the most serious stages of illness or injury, as it is widely acknowledged that by stimulating the body to behave involuntarily by gasp reflex, the innate survival self-healing powers of the human body can be provoked.

By the early 1960s cold application techniques were advancing and became used at the Brook Army Hospital during the rehabilitation of patients with acute sprains, strains and contusions. The first reports from the Brooks group appeared in 1964 from Grant and Hayden (1964), who were a physician-physical therapist team. They described the treatment of soldiers, who had suffered assorted musculoskeletal injuries during basic training, with a new method that they termed cryo-kinetics, which refers to a combination of cold water and exercise. It is here that they used ice massage or ice immersion, depending on the area of the body.

By the early of 1970s cold was used almost universally by sport medicine practitioners, in the care of sports injuries and recovery for immediate care, but not by the medical community as a whole (Barnes.,1979). Subsequently, during the 1970s and 1980s, efforts were made to expand the theoretical basis for the use of cold application in order to understand the benefits of the cold and when it should be applied. In fact, it was theorised that the reason for using cold water was that it can decrease blood flow and thereby decrease swelling (Knight.,1995).

In regards to the utilisation of cold, Cryotherapy is commonly defined as a therapeutic application and it is used to describe the act of using cold temperatures to treat an injury (Goodall and Howatson, 2008). Knight (1995) also defined Cryotherapy as the therapeutic application of any substance to the body that would result in the withdrawal of heat from the body, and thereby, the application of a cooling agent will significantly lower temperatures of the skin and underlying muscle tissue.

Cryotherapy is used widely to treat acute traumatic injury and as post exercise recovery modalities after training and competition that cause some level of traumatic injury (Vaile et al., 2008; Burnett et al., 2010). The term Cryotherapy, as described by Knight (1995) is an umbrella that covers a number of specific techniques, which have numerous methods of application. These include the application of ice packs, ice massage, crushed ice, whole-body Cryotherapy and Cold-water immersion (CWI).

The definition of Cold-water immersion is when the water temperature is less than 15°C, and is dependant on the temperature at which the pain induced by the cold began (Bleakley and Davison, 2010). This form of treatment gained popularity as a recovery modality in sport in the late nineties, thus inspiring many fellow sports therapists to conduct further investigations and hypotheses in this field. Eston and Peter (1999, p 232) state, "as far as we are aware, this form of Cryotherapy has not been used in human studies to assess the effects on muscle damage resulting from exercise". Since then, Cold-water immersion has been frequently used in sports medicine, particularly among high-level athletes, in an effort to minimize DOMS (Sellwood et al., 2007). However, the rational use of ice water immersion in sport appeared from the need of athletes to hasten a return to their optimal performance capability. Furthermore, anecdotal reports indicate that Cold-water immersion may have a positive impact on muscle soreness after an intense or unaccustomed training session, which may help athletes to continue training at peak intensity over subsequent days (Eston and Peter.,1995; Sellwood et al., 2007).

3.2 Mechanisms of water immersion during recovery

3.2.1 Hydrostatic Pressure

When a person is immersed, water exerts a compressive force on the body called hydrostatic pressure. As a result of the pressure, the displacement of fluids from the extremities toward central cavity occurs (Wilcock et al., 2006).

The magnitude of this pressure is increased in relation to the depth of submersion and is described by the equation:

$$P = P_{atm} + g \bullet p \bullet h$$

Where P = water pressure, P_{atm} = atmospheric pressure (standard sea level 1013 hPa), g =gravity (9.81m.s^{-2}), P = water density (1000 kg.m^{-3}) and h = water height (Wilcook et al, 2006).

Proportional changes such as these, being at a depth of 1 meter, which equate to 74mmHg in pressure, have been known to cause an upward directional suction motion to the body (Figure 3.1).

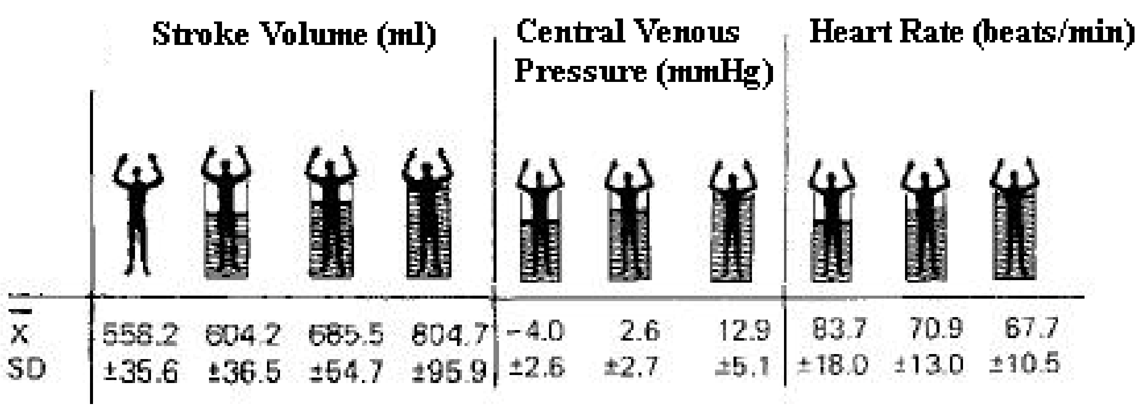


Figure 3. 1 Increase in stroke volume, central venous pressure and decrease in heart rate with increasing immersion depth. Adapted from (Risch et al., 1978).

It is believed that as the level of immersion depth is increased, the central venous pressure increases respectively. However, increasing the external pressure on the body has been shown to have an alternative effect (Löllgen et al., 1981). This is because fluids and gases are emitted, thus causing a displacement from high-pressure areas to lower levels (Risch et al., 1978). The accumulation of fluid from the extra-vascular space moves into the vascular compartment, reducing exercise-induced muscle volume (Wilcock et al., 2006). Substances of gas and fluid are displaced to lower pressure areas through an increase in external pressure on the body (Bove, 2002; Farhi & Linnarsson, 1977; Lollgen et al., 1981). Hence, individuals experience compression on their bodies that acts inwards and upwards when they stand in water. However, when immersion to a person's hip-level is undertaken the displacement of fluid into the thoracic region from the lower extremities is caused by the action of "squeezing". Additionally, hydrostatic pressure upon the central cavity when only head-out immersion is set in place creates a reduction in the lungs' residual air volume, which increases fluid displacement into the thorax region (Farhi & Linnarsson, 1977). Consequently, an athlete's ability to recover from strenuous exercise may be enhanced by the movement of these fluids.

Increased external pressure causes a shift of fluids from the lower limbs into the thoracic region, as indicated by an increase in stroke volume and overall blood flow (Risch et al., 1978; Lollgen et al., 1981). The increase is proportional to the depth of immersion, with greater changes seen as the depth of immersion increases.

Hydrostatic pressure plays an important role in the recovery process of the reduction of oedema (Stocks et al., 2004). Apart from assisting in the possible removal of substances, the gradient between internal tissue hydrostatic pressure and capillary filtration pressure may also improve the reabsorption of interstitial fluids, which will reduce oedema. The recovery process can be assisted by water immersion through increasing the pressure gradient between the interstitial compartment of the legs and the intravascular space (Wilcock et al., 2006). Afterward, the type of swelling that occurs is dependent on the space available for accumulated swelling, which in turn can typically reduce itself due to the lack of space to accommodate progressive or extensive swelling (Kraemer et al., 2004).

Oedema is a condition that can occur following exercised induced muscle injury and can dramatically reduce the required levels of oxygen needed for local cells. As a result this can cause cellular damage and increase the transportation route of localised capillaries, causing internal compression (Wilcock et al., 2006). Oedema can also infiltrate increased levels of leukocytes and monocytes. In addition to this, oedema can circulate an increased flow of inflammatory cells, causing further tissue degradation; namely secondary muscle damage (Cesari et al., 2004).

With the use of hydrostatic pressure, potentially harmful factors can be combated, or indeed avoided, as hydrostatic pressure is known for its anti-inflammatory properties in aiding the clearance of inflammatory mediators from the damaged areas .It is believed that temperature and pressure induced changes in blood flow and reduce muscle temperature that subsequently reduce post-exercise inflammation (Grgeson et al., 2011; Wilcock et al., 2006). Thus, by decreasing the presence of oedema the inflammation can be reduced and further promote the physiological processes needed for a speedy recovery from injury (Sayers et al., 2000). Without the use of preventative intervention from such methods as hydrostatic pressure, the formation of oedema can have a considerably negative impact on any chance of a speedy recovery from injury (Kraemer et al., 2004).

3.3 Literature Review

3.3.1 Physiological effects of Cryotherapy

It has been suggested that CWI has a number of acute physiological effects, namely metabolic, hormonal and also thermoregulatory responses (Halson et al., 2008). The changes in the cardiovascular system, though, is one of the most investigated topics, mostly due to the reductions in heart rate and cardiac output, as well as an increase in arterial blood pressure and peripheral resistance being documented (Wilcock et al., 2006).

Immersing in cold water can have an impact on the human body by exerting both a stimulation of baro and cold receptors, and also activating the nervous system and endocrine functions (Cuesta-Vargas et al., 2013). Subsequently, responses such as these may influence the mechanisms responsible for controlling the cardiovascular function and water balance (Šrámek et al., 2000).

Rapid immersion of humans into water temperatures of 14°C induces a shivering response from the body. In such instances, the shivering motion can also be regarded as a form of light exercise experience for the body (Christie et al., 1990). In fact, the action of shivering is the human body's instinctual action that occurs for the sole purpose of re-generating heat loss, in order to reduce or cease the level of core cooling (Tikuisis et al., 2002). Shivering not only disturbs the boundary layer, but also facilitates heat loss by increasing muscle perfusion. Thus, if the water temperature is low enough, then a slight elevation in shivering may actually increase heat loss more than heat production (Stocks et al., 2001). The immersion of one's body in cold water can cause sudden stimulation to the peripheral cutaneous cold receptors, which produces a rapid and fast fall in skin temperature. This in turn evokes the initial physiological responses to cold immersion, which is given the generic name "cold shock" response (Tipton et al., 1998).

Previous studies have shown that the cold shock response begins from water temperatures below 25ºC (Keatinge and Nadel, 1965) and peaks at a temperature between 10-15ºC (Tipton et al., 1991). Therefore, the most serious shock response by the body is usually stimulated at lower temperatures. Additionally, all other physiological changes occur in response to low temperatures (Seo et al., 2013). These include a "respiratory gasp" reflex, which is comprised of cold shock followed by a short period of uncontrollable hyperventilation and tachycardia, hypocapnia, peripheral vasoconstriction, and hypertension. Within 30 seconds of immersion the response will reach a peak and then readapt within the first 3 minutes in most individuals (Datta and Tipton, 2006). Consequently, it is during this heightened reactionary period of cardiorespiratory and vascular responses that will indicate the initiation of the muscle soreness reduction by CWI. Accordingly, it is necessary to comprehend the overall physiological changes to the body caused by CWI.

3.3.1.1 Temperature

The initial physiological response of human tissue to cold water occurs when heat is withdrawn from the body, which thereby lowering tissue temperature (Stocks et al, 2001). As cooling decreases, tissue temperatures indirectly provoke the sensory nerve endings to the epidermis, which are persistently released until a state of physiological exhaustion (Stacey et al., 2010). In most instances, this action causes a subject to experience a sequence of anticipated sensations, which may result in sensations, such as aching or burning; both of which eventually lead to a numbing sensation (Cataldi et al., 2013).

During cold immersion, skin temperature rapidly falls towards the experienced water temperature, and as a result, more rapid heat loss occurs through the process. In fact, the core temperature falls two-five times more quickly compared with that observed in air at the same temperature (Stocks et al., 2001).

A study was conducted by Hayward et al. (1977) that included eight active athletic male subjects, and was designed to obtain the necessary data regarding adult male humans to derive the three constants: reference core temperature, reference skin temperature and to observe the thermoregulatory heat production in man following CWI. The subjects wore bathing trunks and were immersed in a tank (2 metres (m) deep, 2 m long and 1 m wide) at a temperature of 10° Celsius (C). Subjects sat in a chair and were instructed to remain still with no voluntary activity and to not consciously attempt to influence their amount of shivering. The subjects exited the cold water when their core temperature had declined to approximately 35°C. Then the subjects were transferred to a hydrotherapy bath of rapidly-circulating water, which was 27°C before it was raised over the next eight minutes to 40° C. The subjects' temperature and metabolic rate were recorded throughout, from the stage of pre-immersion up to 30 minutes after re-warming. The author observed that skin temperature decreased in the process from the pre-immersion level of 33.5 ± 0.3° C to an almost constant level of 12.8 ± 0.3° C. However, the results by Howard report the decrease of the core temperature failed to report the accurate time occurrence of decreased temperature, which makes it challenging to estimate definitive immersion time periods that are required to lower the core body temperature. Nonetheless, the current study does not incorporate a greater understanding through measurements of the most productive temperatures, as its focus is purely on the time period changes in CWI.

Separately, a study by Rupp et al. (2012) randomly assigned 18 healthy males to either a crushed-ice bath or cold-water immersion until their intramuscular temperature decreased 8°C from the baseline. The results asserted that intramuscular temperature becomes significantly decreased and stays colder after 90 minutes following cold-water immersion. In addition, it is confirmed that rectal temperature shows a steady decline during a 20 minute immersion period at 13°C, which is due to "local cooling in the rectum due to a return of cooled blood from the legs, rather than the indication of true core temperature" (Martin et al., 1977: 214). Similarly, Sramek et al. (2000) conducted a study of 10 young men, who had to undergo a one hour head-out of water immersion session while sitting on a chair in waters of different temperatures (14°C, 20°C, 32°C). The data indicates that body temperature decreases by approximately 1.7°C at 14°C and rectal temperature becomes lowered from 37.3°C to 35.6°C. Likewise, at 20°C rectal temperature decreases from 37.1°C to 36.1°C, whereas in contrast at 32°C the rectal temperature does not change. In addition, (Johnson et al., 1979) confirm that after 15 minutes of immersion, the rectal temperature and tympanic temperature began to decrease at a steady rate when the subjects were immersed at 10° C. Therefore, it is possible to theorise the exact temperature and immersion periods that would provide the most beneficial effects upon muscles soreness and deterioration by CWI. However, it is relevant to state that the longer implemented duration periods at lower temperature in the studies by Rupp and Sramek may become intolerable to the participants. The effect of CWI on muscle soreness is temperature dependent. Therefore, it is beneficial to estimate definitive duration time periods that can cause a physiological changes.

3.3.1.2 Heart Rate

The increased localized vasoconstriction caused by cold-water therapy results in increased peripheral and arterial resistance, which leads to a higher heart rate (HR) and cardiac output (Petersen et al., 1992; Park et al., 1999; Šrámek et al., 2000). Furthermore, increased central volume and faster heart rate recovery, as well as the parasympathetic activity induced by CWI may contribute to enhanced functional recovery (Haddad *et al.*, 2010; Buchheit *et al.*, 2008).

A study by (Cooper et al., 1976) reports that HR increases approximately 20 beats/ minute (min) when subjects are immersed in water at 10°C compared to immersion at 27° C. Additionally, Keatinge and Evans (1961: 39) conducted a study of twelve unclothed men, who were immersed in cold water (5°, 15° C) for 20 and 40 minutes. From the findings it was stated that the reason for an early increase in HR could be due to “the intense sensation of cold causing reflex release of adrenaline and noradrenaline into the general circulation and into the vicinity of the pacemaker of the heart”. These individual studies used active participants who were immersed in different water temperatures, which both resulted in an increase of HR, post-immersion. Therefore, it is possible to implement a reference point in the current study of the temperature correlation to HR increase, which will determine the most effectual body response to CWI.

On the other hand, when a different study was conducted with practising athletes contradictory results were ascertained (Janský et al., 1996). Twelve male athletes underwent head-out water immersion (14°C) while sitting for one hour, with a three times a week repetition of the cooling procedure for a six-week period, and it was determined that no changes occurred in the HR as a result of cold immersion.

Separately, an investigation by Crowe et al. (2007) noted that HR was significantly lower in the CWI group compared to control group post 30 min cycling test. 17 active healthy subject underwent seated CWI up to the level of the umbilicus at 13-14°C for 15 minute. The same results were reported by Schniepp et al. (2002), as ten well trained cyclists underwent 15 minute CWI at 12°C up to either the iliac crest level or in a seated position, while participants performed 2 maximum effort sprints separated with intervention. HR was found to have a significant decrease form trail 1 to trial 2 post CWI compared to the controlled condition ($P < 0.01$).

In can be concluded that, even though cardiovascular function that is altered by CWI may be theoretically beneficial, especially between bouts of exercise, the significance of these changes with respect to recovery of muscle performance remains unknown. In accordance, the studies that concluded different effects of CWI upon HR used the same position (seated), but with different temperature rates, which could have produced the contradictory results. Likewise, the study by Jansky et al. (1996) utilised repeated immersion that could have affected the HR differently in comparison to the alternative studies that also included pre-CWI exercise. Therefore, the current study analyses both active individuals and elite athletes in order to produce results to show the comparative effects of CWI for both sets of individuals.

3.3.1.3 Blood Pressure

Beside vasoconstriction, which is mediated by sympathetic nerves releasing norepinephrine, the CWI also has a direct impact on blood pressure. A study by Jansky et al (1996), as mentioned previously, examined ten young athletes, who underwent head-out water immersion at 14° C while sitting in a chair for an hour, with a three times a week repetition of the cooling procedure for the duration of six weeks. The heart rate, blood pressure and temperature were measured throughout the research period, which resulted in researchers observing that systolic and diastolic blood pressure were increased by approximately 10% during cold-water immersion.

In the study by Sramek et al (2000) of ten young men who were immersed for one hour with the head out in different water temperatures: 14°, 20° and 32°C, it is shown that cold-water immersion at 14°C increases systolic pressure from 113 to 121, and diastolic blood pressure from 77 to 83. Simultaneously, the study by Jansky et al. (2006), which was previously described in detail, indicates a temporary increase in systolic and diastolic blood pressure within the first 8 minutes of immersion at 12°C. However, Sramek et al (2000) state that at 20° and 32° blood pressure becomes lowered, although no factual score indication from the study is presented.

Most of the studies that have investigated the physiological changes to the temperature, heart rate and blood pressure after cold-water immersion were conducted ten or more years ago (Kowal, 1983; Strong et al., 1985; Tipton., 1989; Tipton et al., 1991; Jansky et al., 1996; Swenson et al., 1996; Park et al., 1999; Sramek et al., 2000). Hence, the results from these studies may remain inconclusive to present research studies, as they did not incorporate techniques such as repeated immersion. Nevertheless, some recent studies have explored the physiological changes to the body with repeated immersion and adaptation (Vybíral et al., 2000; Jansky et al., 2006). However, habituation often influences the overall results, which is why the current study focuses on and investigates the first reaction to CWI.

3.3.1.4 Respiratory Response

Cold-water immersion provokes what is referred to as an "inspiratory gasp". This experience has been documented on subjects, where the gasping reflex action is timely followed by uncontrollable hyperventilation, which is indicated by a dramatic fall in the end-tidal partial pressure and arterial tension of carbon dioxide (Evans, 1961; Golden and Tipton, 1988; Keatinge and; Tipton, 1989)

This inspiratory gasp has been known to occur instantly upon immersion, where the approximately 2–3 litres in volume of lung capacity corresponds with an inspiratory shift in end-expiratory lung volume, which causes the subject to experience uncontrollable hyperventilation (Park et al., 1999). The results of the study by Keating and Evans (1961) of twelve unclothed men, who were immersed for 20 or 40 minutes in temperatures of 5° or 15°C, show that the increase in ventilation in cold water began so rapidly after immersion that it must have been caused by a reflex from receptors in the skin rather than by a change in deep body temperature. However, it may have been due to a reflex directly affecting the respiratory centre, or indirectly through vascular pressure receptors. Meanwhile, the reflex release of adrenaline and noradrenaline probably contributed to the ventilation within a short time after immersion, as these hormones are likely to be released on immersion and consequently cause hyperventilation (Keatinge and Evans., 1961).

The method by which individuals enter cold water has a significant effect on the pattern of respiratory responses, as the cold shock response shows both spatial and temporal summation (Datta and Tipton, 2006). According to a study conducted by Tipton et al, (1998) respiratory frequency increased in the first 30 seconds, when the right side of seven subjects' bodies were immersed in cold water at 10°C for three minute. Furthermore, the findings indicate that elevation of response was reduced over the remaining 2.5 minutes of immersion but still remained above the pre-immersion.

3.3.1.5 Hormones Response

It is generally believed that prolonged exposure to cold is a powerful stimulus for hormonal secretion in humans (Johnson et al., 1976). Several previous studies have demonstrated that exposure to cold air rapidly increase human noradrenaline secretion (Wilkerson et al., 1974; Greenleaf et al., 1980; O'malley et al., 1984;). (Leppäluoto et al., 2005) also state that exposure of healthy subjects to cold air or water leads to a two-five times increase in plasma or urinary noradrenaline levels, however, the hormonal response to cold exposure only lasts for the duration of a few minutes. Nonetheless, some researchers have confirmed that some hormone levels begin to rise within a few minutes after cold exposure or cold-water immersion (Tipton., 1989; Weller et al., 1998). Above all, these results reflect the contrasting results in relation to cold-air exposure and cold-water immersion.

A study conducted by Johnson et al. (1977) reported that the plasma noradrenaline levels had almost doubled after 2 minutes when six subjects were immersed in water at 10° C. Additionally, authors Le Blanc et al. (1979); Hull et al. (1984) both noticed a significant increase in plasma levels of noradrenaline and adrenaline after 1-2 min when one hand or one foot is placed in water at 0-5°C. Also, Smith et al (1990) highlight that noradrenaline and adrenaline increase after cold-air exposure and cold-water immersion, which lasts for 42-45 minutes. However, this evidence suggests that the sudden immersion in cold water activates the sympathetic nervous system, which in turn, rapidly increases noradrenaline plasma levels (Jansky et al., 2006). This fast increase in serum noradrenaline concentration in response to the cold is useful for the heat balance of a body because it causes vasoconstriction, which in turn leads to a decrease in skin temperatures and thus decreases heat loss from the body (Leppäluoto et al., 1988).

Furthermore, catecholomines show the same response to cold exposure as the other hormone, as it increases within 2 minutes of cold exposure (Leppäluoto et al*.,* 2005). Cortisol is usually not secreted in response to cold exposure in humans (Wilson et al., 1970), but secretion increases if the exposure is experienced as stressful (Leppäluoto et al., 1988). Nevertheless, there are some contradictory results regarding the cortisol response.

A review by Bleakly and Davison (2009, p184) confirmed that "A longer 5 min immersion also resulted in significant increases in cortisol levels ($p<0.05$) 30 min after immersion, which returned to resting levels after 1 h". Further studies by Šrámek et al. (1993) have reported a substantial increase in plasma potassium concentration during cold-water immersion. A possible explanation for this, in which cold-induced vasoconstriction is caused in cutaneous veins, has been associated with inhibition of Na+ (sodium), K+- (potassium), ATPase, (adenosinetriphosphatase). In fact, the inhibition of the Na+, K+, ATPase and the subsequent shift of extracellular water into cells may result in haemoconcentration.

Unfortunately, however, at this present time there is no available body of research that can indicate a measured hormonal response through cold-water immersion at the stage of rest without exercise participation.

3.4 Effect of utilising cold water as an intervention on athletes

While DOMS is not a disease or disorder, it can be painful which makes it a valid concern for athletes, as it can limit further exercise in the days following initial training (Sethi., 2012). There are numerous characteristics of delayed onset muscle soreness beyond local muscle pain, which develop about 24 to 48 hours after the exercise activity. The typical symptoms are tenderness with palpation, a decreased Range of Motion, and a muscle strength deficit which may take up to two weeks for maximum recovery (Cleak and Eston., 1992; Curtis et al., 2010). Moreover, a reduction in muscle force output by as much as 50% has been reported with DOMS (Davies and White, 1981; Newham et al., 1987; Cleak and Eston., 1992). Conjoined, all of these symptoms have important implications in the performance of athletes (Kuligowski et al., 1998).

In order to produce a result which is substantially more clinically beneficial, many researchers have conducted studies on athletes to investigate the effect of cold-water immersion on all the symptoms that were associated with soreness and how these have an impact on performance (King and Duffield, 2009). Some researchers compared two groups of recovery, 'cold water' and 'passive recovery' (Yanagisawa et al., 2003; Bailey et al., 2007; Ingram et al., 2009). Cold water is used as a method of recovery that involves immersing the subject in a cold bath or pool. On the other hand, passive recovery, which is also known as a rest recovery, refers to post-exercise inactivity and the intrinsic return of the body to homeostasis (Sanders, 1996). Studies that have compared the effects of different recovery modes generally have used passive recovery (seated) as a control group. However, the following characteristics discuss the effects of cold-water immersion as a method of recovery in relation to the symptoms of DOMS.

3.4.1.Pain

Muscle soreness is a common effect that is felt by individuals who undertake physical activity to which they are unaccustomed and the level of soreness is known to develop more slowly and peak in the period of 8-24 hours post-activity (Hilbert et al., 2003). It has been proposed that the cold may cause a reduction in pain through several possible mechanisms, namely the inhibition of nocioceptors, reduction in metabolic enzyme activity, reduction in muscle spasms or an altered nerve conduction velocity (Airaksinen et al., (2003); Algafly and George, (2007). However, certain authors attribute this pain perception to the analgesic effects of cooling, rather than an inhibition of muscle damage (Kendall and Eston, 2002; Barnett, 2006; White and Wells, 2013).

There are several studies that present data relating to the effectiveness of CWI on muscle soreness, which are based on different pain measurement methods: visual analogue scores (VAS), Likert Scales, or an Algometer. However, there have been different effectiveness levels of CWI on soreness through each study and method, as shown in Table 3.1 below.

Author	Participants	Ex's used to cause DOMS	Methods of CWI	Measurement	Pain measurement methods	Effect of CWI on Soreness
Bailey et al (2007)	20 healthy volunteer	Intermittent shuttle run	10 min CWI at 10° C	Perceived soreness, muscle function, intracellular proteins (before, after, 1, 24, 48, 162 h after) (MVC, vertical jump, sprint time)	VAS	Less soreness
Ingram et al (2008)	11 athletes male	80 min team sport, 20 m shuttle run test to exhaustion	2 × 5 min CWI (2.5 min out) at 10 °C	Ck, soreness, sprints	Likert Scale	Less soreness rating
Santos et al (2012)	9 highly train male	Two 90-minute training session	(5±1°C) for 19 min	CK, LDH, perceived pain, muscle strength	VAS	Less pain
Eston & Peters, 1999	15 female volunteer	8 × 5 contractions of elbow flexors	15 min CWI at 15 °C	Elbow angle, CK activity, muscle tenderness, edema, isometric strength	Algometer	No effect
Goodall & Howatson, 2008	18 physical active	100 drop jumps	12 min CWI at 15 °C	MVC, CK, soreness, ROM, girth (every 24 hours for 96 hours)	VAS	No effect
Jackeman et al (2009)	18 female athletes	10 sets of 10 repetition counter movement jump	10 min CWI at 10 °C	CK, Preserved soreness, MVC	VAS	No effect
Sellwood et al., 2007	18 male	Eccentric loading with non-dominant leg	5 °C CWI	Pain, swelling, hop- ping for distance (function), CK	VAS	Increase pain

Table 3.1 Efficacy of CWI for treatment of DOMS (Snyder, 2001).

In the randomised controlled trail conducted by Bailey et al. (2007), which measured the effects of CWI on the indicators of muscle damage following prolonged intermittent exercise, twenty healthy men completed a 90 minutes intermittent shuttle run based on a protocol previously shown to cause soreness and muscle damage (Thompson et al., 1999; Bailey et al., 2003). The participants randomly assigned to receive CWI experienced less soreness compared with a control group. Similarly, in randomised cross-over trail conducted by Ingram et al. (2009), CWI was found to be an effective post-exercise recovery method as eleven athletic males reported less soreness after 80 minutes of stimulated team sports followed by 20 minutes shuttle running to the point of exhaustion. Furthermore, Santos et al., (2012) showed that post exercise cryotherapy reduced the expression of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), preserved pain and preserved muscle strength of the upper limb in Brazilian Jiu-Jitsu competitors after 90-minute training sessions. Therefore, these three studies who beneficial effect of CWI for soreness reduction after extensive physical exertion.

In the study by Bailey et al. (2007), the first group received a 10 minutes cold-water bath of their lower limbs (iliac crest was fully underwater) immediately following the exercise, where the water temperature of 10ºC was maintained by adding crushed ice. Alternatively, the second group did not receive any treatment, and therefore, served as the control group. Participants from both groups rated their whole body soreness using Visual Analogue Scale (VAS) immediately, 5 minutes after, and then 1, 24, 48, 168 hours post-exercise. Moreover, immediately following the performed exercise in the study by Ingram et al. (2009), as well as post-24 hours, participants performed one of the randomly assigned post-exercise recovery procedures for a period of 15 minutes: CWI, Contrast (the repeated alternation of cryotherapy and thermotherapy), or Passive Recovery. Accordingly, all of the interventions were completed from a seated position. Separately, following the first session by Santos et al. (2012), five random subjects were immersed in a pool with ice (5± 1°C) for a period of nineteen minutes, and the remaining participants were allocated to the control group. Through this particular study, in order to indicate their perception of pain, athletes used VAS, and subsequently the pain score was measured pre-training, post-training, post-recovery, and following performing a Judogi Handgrip.

In regards to how CWI was presented to be effectual in reducing soreness levels following strenuous exercise, Bailey et al. (2007) ascertained results that showed muscle soreness to be decreased in the intervention group in the periods of 1, 24, and 48 hours post-exercise ($p < 0.05$), which was in total contrast to the control group. In fact, the results from this study are in line with an earlier study by Yanagisawa et al. (2003, p:1522) that stated, "the cooling group showed a decrease tendency in the muscle soreness level at 24-48 h compared with the control group". Likewise, the results by Ingram et al. (2009) show muscle soreness to be significantly lowered at 24 h ($P< 0.05$) in a CWI group rather than in a control or contrast group. Additionally, Santos et al. (2012) demonstrated results that showed the subjective perception of pain to be lower in the cryotherapy condition than in the control group. Meanwhile, Santos et al. (2012) observed that the reduction of pain is associated with a decrease in skin temperature after exercise.

In addition, in support of previous findings, King and Duffield (2009) also reported lower rating muscle soreness 24 hours following both CWI and contrast-water immersion compared to active recovery. Therefore, CWI or contrast water therapy (Cτ WT) interventions may be preferable following intermittent sprint exercise, due to the reduced perceptions of muscle soreness and physical exertion (King and Duffield, 2009, p:1801). In contrast, results presented by Jackeman et al. (2009) contradicted the above findings.

Eighteen female athletes completed 10 sets of 10 repetition counter-movement jumps to induce muscle damage and were then randomly allocated to a control or treatment group. The treatment group was given a single ten minute bout of lower limb CWI at 10°C to the level of superior iliac crest immediately following the damage-inducing exercise, where the temperature was maintained by the addition of crushed ice. The control group underwent seated rest without any other intervention following the damage-inducing exercise. However, even though the two groups received contrasting treatment, there were no differences in soreness levels between the groups, which conflicts with the studies by Bailey et al. (2007), Ingram et al. (2009) and Santos et al. (2009), possibly because more strenuous exercise was implemented into the study to induce DOMS.

Overall, the results from the effect of CWI on soreness are still controversial and inconclusive, as some of the results showed a positive effect and others reported either negative or no effect (See Table 3.1). The discrepancy in findings may be related to the different exercise intensities, different subject exercise levels, and differently timed treatment. One particular study by Sellwood et al. (2007) implemented a single treatment CWI and others used multiple immersions (Eston and Peter, 1999). In general, athletes and non-athletes may differ in terms of tolerance for pain, which may be an important consideration for the interpretation of data derived from a VAS (Manning, 2002). However, it is also important to measure how an individual's strength is affected, as well as pain reduction.

3.4.2 Strength

Strength is an important measure of athletic performance (Cronin and Hansen, 2005), and

DOMS is more apparent during the process of increasing muscle strength through extensive exercise. Accordingly, muscle damage that can possibly occur during such athletic activities can involve a sensation of soreness. This may be due to the possible physiological damage of the sarcomeres, such as the damage or tearing of the Z-lines. Indeed, this form of damage can lead to a decrease in muscle strength (Behm et al., 2001; Cheung et al., 2003).

Researchers have used a variety of ways to assess the decline in muscular strength and power, which is observed following exercise-induced muscle damage (EIMD) or exhaustive exercise. Additionally, it can be seen that most studies use isometric testing tools, such as isokinetic dynameters or force transducers (Kuligowski et al., 1998; Eston and Peters, 1999; Howatson et al., 2005; Howatson and van Someren, 2005; Wilcock et al., 2006; Sellwood et al., 2007; Bailey et al., 2007; Goodall and Howatson., 2008; Vaile et al*.,* 2008). Hence, there are investigators that have reported the effects of CWI on isometric strength, although certain findings have provided conflicting or inconclusive results. In accordance, a decrease of muscle strength post-CWI has been shown when compared to the passive recovery group (Peiffer et al., 2009) and others have determined that either CWI improved the recovery of strength compared to passive recovery (Bailey et al., 2007; Pournot et al., 2011) or that there were no differences between groups (Sellwood et al., 2007; Ingram et al., 2009; Santos et al., 2012).

In relation to different evidential research that presented results that showed strength to improve through CWI, in a study by Hatzel et al. (2000) the post-CWI effects on isokinetic ankle strength were analysed in 20 physically active male college students. In that particular study, all subjects performed plantar flexion/extension and inversion/eversion at speeds of 60°/s and 120°/s in tests of random order. This was subsequently followed by a 20 minute immersion period in a cooled tank of 10°C. Additionally, in the investigation by Bailey et al. (2007) the influence of CWI on indices of muscle damage was evaluated, as CWI to the level of the anterior superior iliac spine (ASIS) or passive recovery was administered immediately following a 90 minutes intermittent shuttle run protocol.

It can be seen in the study by Hatzel et al. (2000) that MVC was measured immediately post-CWI, which determined that dorsiflexion and plantarflexion strength was higher post-CWI compared to the pre-immersion levels. Likewise, in the study by Bailey et al. (2007), the process included monitoring the rates of perceived exertion (RPE), muscular performance (maximal voluntary contraction of the knee extensors and flexors), and blood variables, which were taken prior to exercise, during recovery, and following recovery for 7 days. The authors observed that CWI facilitated the recovery of isometric knee flexion strength at 24 and 48 h compared to the control group. However, this particular study was conducted on healthy males who were unfamiliar with the test exercise, and thus, the data remains questionable as to whether the response from elite athletes would be the same and have the same effects upon muscle strength.

Comparatively, other studies proved inconclusive to the benefits of CWI upon improved flexion or extension strength. Ingram et al. (2009) compared 15 minute protocols of CWI at 10°C to the umbilicus, with hot/cold contrast immersion and passive rest, as well as at 24 and 48 hours post-exercise in team sport athletes who were familiar with the performance tests. Participants performed 80 minutes of team sport followed by a 20 minutes shuttle run test, where repeated sprint ability, muscle soreness, alongside strength and inflammatory markers were measured across the 48 h post exercise period. Separately, Peiffer et al. (2009) examined the effect of 20 minutes CWI (14°C) on neuromuscular function, rectal temperature and skin temperature, and femoral venous diameter on ten well trained male cyclist following two bouts of a 90 minute cycling session. Additionally, at the stage of 25 minutes post-exercise, Peiffer assigned participants to either the CWI or the passive control recovery group. Moreover, maximum voluntary isometric torques (MVIC) of the knee extensors, and MVIC with superimposed electrical stimulation (SMVIC), were measured prior to exercise, as well as 0, 45 and 90 minutes post-exercise. Furthermore, in the study by Sellwood et al. (2007), the effect of ice-water immersion was investigated on DOMS through shorter immersion periods. Following a leg extension exercise task (5 ×10 sets at 120% concentric 1RM), participants performed 3 × 1 minute water exposure, which were separated by 1 minute in either 5 °C or 24 °C (control) water. Through this procedure, pain, swelling, muscle function (one-leg hop for distance), maximal isometric strength, and serum CK were measured at the baseline, 24, 48, and 72 h post-eccentric exercise protocols.

It was shown in the study by Ingram et al., (2009) that no significant effect of intervention was observed in knee flexion or extension strength, although it was reported that strength losses were observed at 48 hours following contrast and passive recovery, although they were not observed following CWI. Nonetheless, repeated sprint performance demonstrated a more rapid return to the baseline following CWI. Therefore, these results into muscle strength loss are contradictory to Bailey's (2007) findings, although it should be noted that Ingram used multiple intervention periods, which could have a decisive impact upon the changes in results. Moreover, the results from Peiffer et al. (2009) showed greater decrements in MVIC and SMVIC of the CWI group compared with the passive control group. Yet, even though the participants were well-trained cyclist, the measurement was only recorded up to 90 minutes post-exercise. Therefore, it remains unclear whether reduced muscle strength will recover or remain decreased in longer periods of recovery. Likewise, it is inconclusive whether the precise effects of shorter immersion times upon MVIC and SMVIC would be comparative to the results shown in the study by Peiffer et al. (2009). Similarly, as a result of the study by Sellwood et al. (2007), the authors reported that no significant differences were evident for isometric strength between the control or intervention group at any specific time.

In addition, immersion in ice water failed to influence the dynamic strength test in the study carried out by Santos et al (2012), who utilised a cross-over study design, where two training sessions were separated through a two day period. The authors investigated the acute effects of post exercise cryotherapy on the expression of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), as well as the perceived pain and muscle strength of the upper limbs in Brazilian Jiu-Jitsu fighters. Nine highly trained male fighters were subjected to two 90-minute training sessions, after which, five of the participants were randomly chosen to undergo CWI for nineteen minutes. Likewise, the remaining four participants became a separate control group. On the second training day the treatment was reversed for the participating groups, as immediately after the training session the control group of the previous day were immersed in an ice bath (5±1°C) for 19 minutes, which consisted of four cycles of four-minute immersions separated by one-minute intervals. Furthermore, the upper limb strength was measured pre-training and post-recovery through the handgrip test. This process consisted of the athlete performing the isometric test, resting for 15 minutes, which was then followed by a dynamic test.

Santos et al. (2012) clarified that the results were due to the fact that the rest period of 15 minutes was sufficiently long enough to promote full recovery. However, at the same time it was found that even in the control condition, the athletes were entirely recovered prior to the implementation of the dynamic strength test. Additionally, there was no sufficient information regarding the control group's recovery method or isometric and dynamic measurement intervals. Nevertheless, despite insufficient information, the results by Santos et al. (2012) raise questions about the findings of Peiffer et al. (2009), who included strength measurement after a period of 45 minutes. However, there was not enough information relating to the result from the study of Peiffer et al. (2009), except that MVC showed an improvement post-CWI. Moreover, the authors did not provide the statistical analysis of significance for MVC to extract a clear comparison.

In contrast, the results by Pournot et al. (2011) show an improvement in MVC after cryotherapy when they tested 41 highly trained males (Football, Rugby, Volleyball). The subjects performed 20 minutes of exhaustive, intermittent exercise followed by a 15 minutes recovery intervention. Accordingly, the recovery intervention consisted of different water immersion techniques: Temperate-water immersion (36°C; TWI); Cold-water immersion (10°C; CWI); Contrast-water temperature (10-42°C; CWT); and a passive recovery. Through these techniques, counter- movement jump (CMJ) and MVC were recorded pre and post exercise, as well as 1 h and 24 h post intervention. Indeed, it was postulated by Howard et al. (1994) that the performance decrements may be attributed to cold induced effects, such as reduced nerve conduction velocity, cross-bridge deactivation, alterations of the motor unit recruitment order and increased tissue viscosity which leads to added resistance to cross-bridge formation. However, in regards to the results in the study by Pournot et al. (2011), it was demonstrated that a significant increase in MVC one-hour post intervention existed when compared to pre-exercise value; whereas CWT data indicates that a suppressed performance state for MVC is not evident 24 h post exercise. Overall, when the findings of Santos et al. (2012) are analysed and taken into consideration, the results of Pournot et al. (2011) also become questionable regards the improvement of MVC post cryotherapy.

3.4.3 Effect of cold water on inflammatory and muscle damage markers

In the context of the effect of cold water on enzymes, the majority of previous studies have focused on measuring the level of muscle damage blood markers (CK, Lactate, Myoglobin, LDH) and the inflammation blood marker (C-reactive protein CRP) as a secondary outcome (Ascenasaao et al., 2006; Crowe et al., 2006; Draper et al., 2006; Gill et al., 2006; Duffield and Marino, 2007; Baily et al., 2007; Goodall and Howatson., 2008; Ingram et al., 2009; Corbett et al., 2012). Therefore, the outcomes of the common markers that are addressed by the stated authors are presented and discussed in detail below.

3.4.3.1 Creatine kinase

Many researchers consider creatine kinase (CK) concentration as a reliable marker of skeletal muscle damage (Clarkson et al., 1992; Hartmann and Mester, 2000; Cheung et al., 2003; Kobayashi et al., 2005). Even though the large inter-subject variability sometimes makes it difficult to interpret the data (Nosaka and Clarkson., 1996), the variability within a subject is more stable (Clarkson and Sayers., 2007). High intensity exercise that causes damage to the skeletal muscle cell structures at the level of the Z-discs and sarcolemma causes an alteration in membrane permeability and the release of enzymes, such as CK (Noakes, 1987). These enzymes then leak into the interstitial fluid, which is then taken up by the lymphatic system and returned to the blood circulation (Brancaccio et al., 2007).

Previous researchers have hypothesized that the use of CWI post-exercise may alleviate the induced muscle damage inflammatory response, which reduces the efflux of muscle damage markers from skeletal muscle (Eston and Peters, 1999). Additionally, the lower CK concentrations are induced by CWI conditions (Pournot et al., 2011). Consequently, the reduced diffusion rate may assist in reducing acute inflammation from muscle damage and immune activation (Stacy et al., 2010).

Nonetheless, certain studies have contradictory reports on cold-water therapies. Hence, these investigations only offer limited or in fact no beneficial data on the therapies' effects, when it is required to focus on conventional severe single muscle groups, or whole body groups that are being used as exercise subjects. Likewise, a level of controversy is inferred on the matter, as to whether cold water creates an effective response and its impact on the appearance of intracellular proteins in plasma during the time of recovery. Therefore, there have been diverse results regarding the effect of cold water in CK concentration.

Serum CK increases significantly ($p < 0.05$) 48-hours post-exercise period in all recovery conditions, which peaks at 24 hours post-exercise, as shown in the study conducted by Ingram et al (2009). However, they conclude that no significant differences have been found between recovery conditions for muscle damage markers (CK) between the intervention group and the control group. Moreover, (Baily et al., 2007; Jakeman et al., 2009; and Pointon and Duffield, 2012) concur with the pervious result, as both have stated that CK increases after exercise, although no significant differences have been noted after cryotherapy when compared to the control group.

Contrastingly, Pournout et al (2011) has observed that plasma CK only changes through the CWI conditions group compared to passive and temperate water-immersion groups, although the raise in concentration of CK is stunted at 24 hours post-exercise. According to the author, other groups apart from the CWI group showed a significant increase ($P<0.05$) in concentration of plasma CK pre-exercise and 24 hours post-exercise. In addition, Santos et al (2012) note that the level of (CPK) increases significantly ($P=0.05$) for the control group when compared to the cryotherpy group, which indicates that there is less muscle damage after immersion in ice, according to the researchers.

3.4.3.2 Blood Lactate

During the initial phase of carbohydrate metabolism, a metabolite known as lactate is produced to enable the body to endure high intensity activity (Goodwin et al., 2007). In fact, the link between blood lactate and the accumulation of lactic acid was once considered to be a major cause of DOMS. However, it is now known that blood lactate actually returns to resting levels, normally within one hour of performing moderate exercise, and is often shown even after undertaking extremely extensive exercise (Naiya, 2012).

Peripheral vasoconstriction and the associated reduction in blood flow have been reported as negative consequences of CWI. This view is due to the likely repercussions on the clearance of exercise induced metabolites, such as lactate. Through this study, the reduction in blood lactate concentration was slower following CWI than after Active recovery, which may indeed be caused by lower blood flow (Vaile et al., 2010).

Cold-water immersion, though, had suggested to enhance lactate clearance through an increase in blood flow (Peiffer et al., 2009). However, the results of the study by Parouty et al (2010) show that a 5-minute cold-water immersion had no effect on lactate clearance. Crowe et al (2007) measured the blood lactate on 17 (13 male and 4 female) active athletes. They completed two tests in a period of 2 to 6 days apart. These tests comprised of two 30 minutes cycling at maximum effort, which were separated by a one hour recovery period, as well as a 10 minutes cycling cool down followed by passive rest or 15 minutes CWI (13-14°C water). The combination with passive rest after CWI investigated the effects of CWI on recovery from anaerobic cycling. Moreover, peak power, total work and post-exercise blood lactate were measured. The results showed that blood lactate decreases only in the second exercise test following CWI when compared to both the first exercise test by both groups and the controlled group in the second exercise test.

The researchers have explained that the contrasting findings are due to a decrease in immersion time, as fluid shifts occur during immersion as a result of hydrostatic pressure, which can affect blood lactate concentration. Additionally, Valie et al (2008) have stated that no significant differences would be observed in blood lactate concentration between any of the recovery interventions, during exercise. Nevertheless, research into lactate concentration is narrow, thus only a dearth of studies, which had compared the concentration of lactate between a CWI group and a control group, were found in this literature review.

3.4.3.3 Inflammatory Marker

It is clear that inflammatory and muscle damage markers can increase significantly following sport activities (Anderson et al., 2008; Ascensao et al., 2008). Muscle damage can affect muscle strength; also acute inflammation can lead to chronic inflammation. Cold-water immersion may play a role in decreasing the inflammatory and muscle damage markers that are associated with the exercise. Several studies have investigated the effect of CWI on both markers (Rowsell et al., 2009; Lee et al., 2012). Post 90 min of treadmill exercise followed by CWI and passive recovery, the level of IL-6 decreased in CWI compare to the passive group. While different result reported following 40- minutes of cycling, there were similar levels of interleukin-6 (IL-6) and C-reactive protein (CRP) between CWI and control group in the immediate follow-up to exercise (Halson., 2008).

3.5 Athletes' perceptions of CW recovery

A perception is defined by Hochberg (1956) as "Psychophysical scaling of experimental situations in terms of the immediacy or perceptual quality of the experiences they arouse" (p: 404). A study by Cook and Beaven (2013) used a psychological tool that reports to provide valuable information regarding recovery. Thus, the researchers were able to predict the efficacy of the intervention, which contributes a vital part in the players' outcomes.

However, Stanley et al., (2012) state that a subject's belief of the efficacy on an intervention could influence any subsequent response.

In their study, the researchers assessed the perception of cold-water recovery on 18 well trained cyclists, who had completed a one hour high intensity session followed by 10 minutes of the three treatment recovery (Passive rest, cold water immersion, contrast water immersion) (Stanley et al., 2012). This assessment was conducted by the utilization of a psycho-physiological recovery questionnaire, which included: general fatigue, mental recovery, leg soreness and physical. Nonetheless, although both hydrotherapy interventions improved perceptions of recovery, it is likely that these perceptions rapidly diminished after the time trial commenced, as indicated by the similar performance in each trial. Therefore, the psycho-physiological effects of post-exercise hydrotherapy and their relationship to exercise performance warrant further investigation.

The belief of recovery effectiveness and readiness for exercise, pain and fatigue were investigated in a study conducted by Broatch et al. (2014), who examined the physiological merit of CWI for recovery by investigating whether the placebo effect is responsible for any acute performance or psychological benefits on 30 recreationally active healthy men. They performed an acute high-intensity interval training session (HIT), comprised of (4×30s) sprints on a bike followed by one of the following three 15 minute recovery conditions: an ice bath (10°C), a warm bath control (34.7°C), a placebo (34.7°C). Furthermore, muscle temperature, thigh girth, pain threshold and tolerance, MVC, IL6 were recorded at baseline, post-exercise, post-recovery, as well as 1, 24 and 48 h post-immersion. Likewise, questionnaires were distributed prior to HIT and recovery protocols in order to measure the anticipated effectiveness of the assigned recovery techniques. Moreover, a psychological questionnaire was incorporated to provide a rating of the participants' readiness to exercise, fatigue, sleeping patterns and muscle pain. Consequently, the results determined that both CWI and TWP (thermoneutral water immersion placebo) were superior to a TWI (thermoneutral water immersion) condition in assisting the recovery of the quadriceps strength after an acute HIT session. Therefore, the author concluded that a strong belief in ice baths combined with any potential physiological benefits could maximize the potential enhancement of an athlete's recovery from exercise.

Even though the study by Broatch et al. (2014) presented a conclusion of the benefits of a positive psychological approach to CWI, there is presently a distinct lack of evidence regarding participant expectations into the efficacy of cold-water modality. Likewise, Cook and Beaven (2013) have emphasized that expectation is considered to be a significant contribution to both positive and negative sports performance.

3.6 Conclusion

The benefits of the cold have been appreciated for many thousands of years. Wang et al (2006) state, "The use of cold as a therapeutic agent has a long and colourful history" (p565). More specifically, it has a long and popular history of being used as a standard treatment for soft-tissue injury, which has been the pivotal essence of CWI therapy. In fact, the reason for CWI gaining popularity is mostly down to its analgesic effects, as well as for the anti-inflammatory properties that the cold has shown to offer (Cooper and Dawber, 2001; Damijan and Uhryński, 2012).

There are physiological changes that have been associated with CWI, and its practice has documented data that shows increases in heart rate, blood pressure, respiratory minute volume and metabolism. Most of the documented studies that explain the physiological changes to the temperature, heart rate and blood pressure in rest position, all began ten years ago. Moreover, the majority of the research has investigated these physiological changes during the process of exercise activities (Bleakley et al., 2010; Leeder et al., 2011; Bleakley et al., 2012). Therefore, it can be agreed that CWI has shown to have beneficial and therapeutic effects on the body, but further investigation and evaluation to the physiological effects of the cold on the body in the period of rest is required. The cold can have an effect greater than simply a placebo, or subjective improvement in recovery and performance ability.

Nonetheless, at present there are conflictive or inconclusive results on the effects of CWI in attenuating the muscle damage post-EMID. This conflict is highlighted particularly in isometric strength, which has shown to have a direct impact on overall performance. However, it is agreed that CWI has provided an effective part in reducing the concentration of CK following EMID.

Methodological differences between each investigation into CWI have reported inconsistent results. These contrasts are usually found in literature, such as unspecified observations of single muscle groups or whole body groups, and have tended to draw up inconclusive or conflictive findings. Furthermore, the lack of literature available on an athlete's overall personal expectations and experiences of CWI can lead to an unclear decision when exacting any physiological outcomes, and whether there are beneficial results of cold water.

Chapter Four: Methodology

4.1 Introduction

This section describes the aims and objectives as well as the procedures used within the research. A mixed-methods research design was used to address the aims and objectives of this research programme. This included quantitative physiological measurements in an experimental setting as well as qualitative surveys of the perceived and psychological effects of CWI therapy.

4.2 Aims and Objectives

The overall aim of the work reported in this thesis was to determine the effectiveness of *cold- water immersion* (CWI) as a therapeutic strategy to improve recovery from intense, muscle damaging exercise. There were three main objectives:

1. to examine cardio-pulmonary, metabolic and neuromuscular responses of young, healthy men to a single 15 min bout of CWI.
2. to investigate whether a single 15 min bout of CWI coming a few minutes after intense, muscle damaging exercise would improve the recovery of skeletal muscle function during the two days following the initial muscle-damaging exercise in very well trained rugby players.
3. to survey the athlete's perceptions of participating in CWI therapy.

4.3 Methods

4.3.1 Research Design

Studies were designed in three *Phases*. The first and second phases were quantitative investigations and the third was qualitative by design. Detailed descriptions of the study phases are provided below. Briefly, in *Phase One*, cardiopulmonary function was measured before and during immersion in a water bath set at 12-13 ^{0}C for 15 minutes. Neuromuscular function was assessed before and after the cold-water immersion. In *Phase Two*, a single-blind randomized controlled trial with a cross-over design was used to assess the efficacy of CWI to improve recovery of neuromuscular function following muscle-damaging exercise. *Phase Three* of the study utilised a focus group interview and questionnaire in order to capture any perceived benefits beyond what could be measured in the quantitative neuromuscular assessments.

4.3.2 Participants

The study protocol was approved by the Research Ethics Committee at Manchester Metropolitan University (Faculty of Science and Engineering) (Appendix 1). Prior to participation, the experimental aims, procedures and potential benefits and risks were explained verbally and in writing to each participant (Appendix 3), followed by completion of a health-screening questionnaire (Appendix 4) and a written informed consent form (Appendix 5).

4.3.2.1 Phase One

Participants were volunteers recruited from amongst the student population at Manchester Metropolitan University using flyers and posters (Appendix 2). Potential participants could be included in the study if:

- aged between 18–40 years old
- active in any recreational sports activities.
- willing to provide blood samples using venepuncture
- healthy, with no history of cardiovascular, neurological or skeletal muscle disorders, muscle or skeletal trauma injury or cold insensitivity.

Nine healthy, recreationally active men met the above criteria and participated in the study, although none were engaged in competitive sports. Their characteristics are presented in Table 4.1.

Subject	Age (Year)	Height (cm)	Body mass (kg)
Mean (± SD)	23.2 (6.07)	177.5 (4.06)	74.6 (9.16)

Table 4. 1 **Physical characteristics of Participants.**

4.3.2.2 Phase Two

Eight professional rugby players were recruited from Rygbi Gogledd Cymru (RGC), Wales, UK. All competed on the North Wales Rugby team and practiced 4–6 hours per day, five days a week. A written description of the overall aims of the study and research protocols were sent to the participants prior to their attendance at the research laboratory (Appendix 6). The characteristics of the participants from Phase 2 are displayed in Table 4.2.

Subject	Age (Year)	Height (cm)	Body mass (kg)
Mean (± SD)	19.25 (2.18)	182.75 (4.92)	95.17 (10.94)

Table 4. 2 **Physical characteristics of Phase 2 participants.**

4.4 Experiment Design

4.4.1 Place of the study

The study took place in the research laboratory in the Faculty of Science and Engineering, Manchester Metropolitan University. The facilities enabled blood sampling, neuromuscular and cardiorespiratory assessments as well as exercise and CWI which were not all possible in field-based assessments.

4.4.2 Procedures

4.4.2.1 Phase One

Phase One was designed to investigate physiological responses to a single acute exposure to cold water immersion, without any prior muscle damaging exercise. A detailed overview of the individual assessments is provided below and the outline is provided here. Participants reported to the research laboratory in the morning after an overnight fast of at least eight hours and having not completed any strenuous exercise for at least 48 hours. Height and weight were recorded, followed by a phlebotomist collecting a blood sample from a vein in the forearm. Neuromuscular function of the right knee extensors was assessed using a combination of voluntary and electrically stimulated contractions on a custom-made isometric dynamometer and on a Cybex dynamometer. The results were recorded in digital format and on a written case report form (Appendix 7). After a 20 min rest, participants sat on a standard chair and for the next 10 minutes cardiopulmonary and metabolic assessments were recorded, including blood pressure, heart rate, breathing frequency, minute ventilation, pulmonary expired oxygen and carbon dioxide. Participants transferred themselves gently into a water bath which was located immediately adjacent to the chair. The water was set at 12-13 °C and participants sat immersed up to their waist with legs fully extended. The same cardiopulmonary and metabolic measurements were recorded throughout the 15 min immersion period. After the immersion, participants quickly dried themselves and blood samples were collected (within 5 min of leaving the cold water bath). Neuromuscular function was assessed within 30 min of leaving the water bath. Blood samples and neuromuscular function were collected again 24 hours later.

4.4.2.2 Phase Two

In Phase Two, a single-blind randomized controlled trial with a cross-over design was used to assess the efficacy of CWI to improve recovery following muscle-damaging exercise. Blood samples and neuromuscular function were collected in the rested, baseline state. In order to understand the effectiveness of CWI in DOMS, muscle damage need to be achieved. Therefore, Participants then completed 20 min step-exercise while carrying an additional 16 kg weighted jacket. One leg was chosen at random to always step up onto a platform that was 39 cm high and the other leg was always used to step down from the platform in a controlled manner. The eccentric contraction of the knee extensor muscles during the downward step, as well as the triceps surae of the contralateral limb during the toe-strike landing, were expected to result in muscle damage and delayed-onset muscle soreness. This hypothesis was formulated from the understanding that certain weak sarcomeres become over-stretched in comparison to others during the process of active muscle lengthening (Morgan, 1990). Subsequently, this will continue and the sarcomeres will steadily weaken until no myofilament overlap is present. Additionally, new sarcomeres become over-stretched through repeated eccentric contractions. Following each contraction, various sarcomeres may not be capable of reintegrating, as the muscle becomes too relaxed, and consequently, becomes disrupted (Morgan, 1990).

Immediately after the stepping exercise, participants were randomly assigned to either the experimental condition that included immersion for 15 minutes into a cold-water bath set at 12-13 ^{0}C, or control condition which included 15 min passive seated recovery at room temperature. Blood samples and neuromuscular function were re-tested immediately after the 15 min recovery period and again at 24 and 48 hrs later. Pain experienced in the vastus lateralis of the eccentrically contracted thigh (i.e. the leg that was used to step down from the platform) was measured at rest using a VAS scale and a Pain Pressure Algometer immediately after the recovery method as well as 24 and 48 hours later. For the cross-over part of the study design, participants returned to the laboratory 14 days later to complete the same experimental procedures, except that the leg which was previously randomised for the downward step was this time used to complete the upward stepping action. Thus, the leg that was not previously damaged during exercise would now be exposed to the damaging exercise. This study design reduced possible confounders from the 'repeated-bout' effects in which muscles that were previously damaged in the past few weeks are somewhat protected from further damage or delayed onset muscle soreness (McHugh, 2003). Figure 4.1 illustrates the process of phase one and phase two.

4.4.2.3 Phase Three

Phase Three of the study utilised a focus group interview and questionnaire (Appendix 8, 9 & 10). Participants were asked to answer in their own words a series of questions that were pre-set and asked in the same way for all. The questions were asked within 45 min of completing the muscle-damaging exercise and then again 48 hours later. The rationale for this form of investigation was that benefits of CWI may be perceived by participants beyond what could be measured in the quantitative neuromuscular assessments.

Figure 4. 1Study design of Phase One and Two

4.4.3 Measurements

4.4.3.1 Blood Sample

Blood samples were taken from both the phase one group and phase two during the measurement period. A venous blood sample of ~8 ml was obtained from a vein in the antecubital fossa using a 21 gauge needle and a vacutainer system. Samples were uniquely coded for each participant and were centrifuged within 30 min of collection at 2000 x g to separate the plasma from the remaining blood constituents. Samples were aliquotted and stored at -80 °C until analysis.

4.4.3.2 Blood Pressure and Heart Rate

An automated digital monitor was used (Omron M6, HEM 7001-E) to measure systolic and diastolic blood pressure (BP), as well as the heart rate (HR). A study by (Beevers et al., 2001) has shown that the body's posture may play a role in blood pressure readings. Therefore, all measurements were taken in the seated position.

For resting, baseline measurements, participants sat in a chair with a back support, arms relaxed and both feet resting flat on the floor with legs uncrossed for a period of at least 10 minutes (Vidt et al., 2010). For measurements taken during CWI, participants sat in the water bath with legs fully extended. The arms were relaxed at shoulder height resting along the side of the bath. An appropriate sized BP cuff was placed over the upper part of the left arm, covering the brachial artery. In Phase One, BP and HR were measured at rest, during the first minute of CWI and throughout the CWI period in five-minute intervals. All measurements were recorded on a case report form (Appendix 11). The Omron M6 device was shown to be accurate and reliable according to the validation criteria of BP measurement (Altunkan et al., 2008).

4.4.3.3 Oxygen Consumption (VO_2)

VO_2 and VCO_2 can be measured in expired gasses to indicate the rate of oxygen uptake and carbon dioxide produced, respectively, during metabolic processes. In very light through to intense exercise the VO_2 and VCO_2 are directly proportional to the metabolic rate and level of energy expenditure. When VO_2 reaches its highest point during intense exercise, it is termed the maximal rate of oxygen uptake, or VO_2max (Hawkins et al., 2007). While no voluntary exercise was involved in Phase One, shivering was a possibility of CWI. Shivering involves muscular contractions and therefore increases metabolic rate, energy expenditure and blood flow to the active muscles (Christie et al., 1990). Hence, a Cortex Metalyzer was used to measure VO_2 and VCO_2 in expired air, which is considered a reliable instrument of measuring pulmonary VO_2 (Meyer et al., 2001). Prior to use, the Cortex Metalyzer was calibrated according to manufacturer instructions using 16% O_2 and 5% CO_2 gas concentrations and the volume of gas flow was calibrated using a 3 L syringe (Appendix 12).

After resting in a seated position for at least 15 min, participants wore a mouthpiece connected to a turbine (to measure gas volume) and a sample line (to measure VO_2 and VCO_2). Resting measurements continued for a further 10 min. Participants carefully transferred into the water bath and remained there as relaxed as possible for 15 min. Breathing rate, minute ventilation, VO_2 and VCO_2 were measured breath-by-breath, while HR and BP were measured every 5 min. The respiratory exchange ratio (RER) was calculated as the ratio of carbon dioxide production to oxygen uptake. The data will be presented of 30 min average over the 15 min.

4.4.3.4 Testing of muscle strength and contractile characteristics

All measurements taken were of the knee extensor muscles. A Cybex II Isokinetic dynamometer (Lumex Ronkonkoma, New York) was used to assess the force-velocity relationship during dynamic knee extensions. A custom-built isometric knee extension dynamometer was used to assess muscle contractile properties using a combination of voluntary and electrically-stimulated contractions.

4.4.3.4.1 Knee extensor force-velocity and power measurements (Dynamic Contraction) (Cybex)

Maximal isometric knee extensor torque and concentric dynamic knee extensor torque at a series of velocities were measured (Schwartz et al, 2010). The Cybex II Isokinetic dynamometers allow the velocity of knee extension to be fixed and for the torque to be continuously measured (Alangari and Al-Hazzaa, 2004). Prior to each test, calibration of the dynamometer were made and adjustments to fit each individual participant. The hip was flexed at 85^0, the lateral femoral condyle of the knee was aligned with the axis of rotation of the lever arm and the part of the lever arm against which the participant should push maximally was positioned on the anterior part of the lower leg around 2 cm proximal to the ankle malleolus (Appendix 13). Stabilization straps were secured around the waist, and upper body to prevent extraneous joint movements and to standardize the body's position. The participants were instructed to place their arms crossed over their chests and to breathe normally throughout the testing procedures. Figure 4.2 illustrates the dynamometer and participant position.

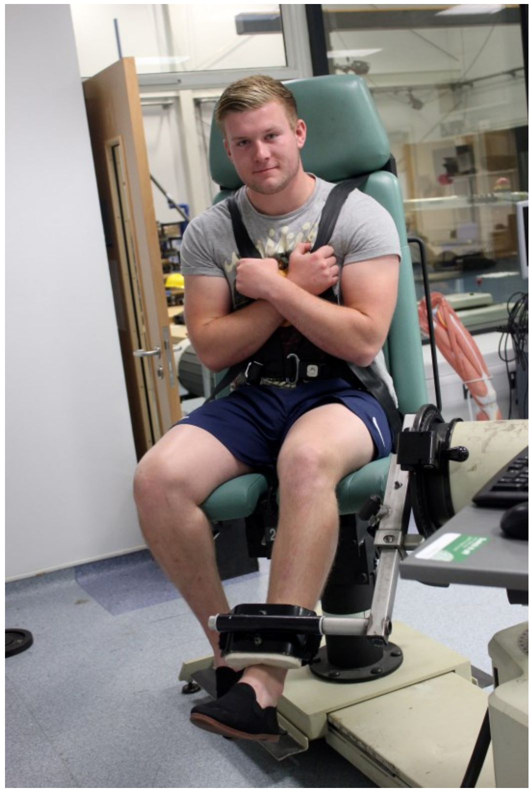


Figure 4. 2 **The Cybex dynamometer and participant position.**

The same researcher was responsible for testing all participants and standardized instructions and encouragement were provided. Prior to each test session, participants performed six consecutive sub-maximal, dynamic knee extensions as a warm-up.

Anatomical zero was set at a knee angle of 0° (full extension). The maximal voluntary isometric contraction torque (MVC) was determined by setting the joint angle at 90° of flexion and then locking the lever arm in this position. MVC was tested with three repetitions, each lasting 2-4 sec with a rest-interval of 1 min. The peak torque (Nm) was taken as the highest value achieved. Following the MVC test, a series of dynamic contractions were used to determine the force-velocity and power relationships of the knee extensors muscle group. The range of motion was set between knee joint angles of 110° (almost maximal knee flexion) through to 20° (almost full extension). Maximal torque was subsequently measured at angular velocities of 0 deg/s (isometric at 90° knee angle), 60, 120, 180, 240, 300, 360 and 420 deg/s (concentric), with two efforts at each velocity separated by a rest interval of at least 30 sec between efforts. The sequence of testing speeds was randomized and a rest of two minutes was permitted between the tests at each velocity. This range of velocities covers slow, medium and fast speeds (Brown., 2000). Isokinetic dynamometry has high intraclass correlation coefficients (ICC) ranging between 0.85 and 0.99, according to (Karatas et al., 2002; Lenaerts et al., 2001).

4.4.3.4.2 Force/frequency relationship

A combination of voluntary and electrically-evoked contractions was used to assess the knee extensor muscle contractile properties.

4.4.3.4.2.1 Electrical Stimulation Setup

The Digitimer high voltage stimulator model DS7AH (Welywyn Garden City, Hertfordshire, UK) was used in this study.

The voltage was set at 400 mA and the pulse width at 200 µs. These parameters were standard for all participants. Two self-adhering stimulation electrode Galvanic pads (AmericanImex, CA, USA: 4x7inches) were used with each participant. The proximal electrode was placed over the region of the thigh covering rectus femoris and vastus lateralis, and the distal electrode was placed over the distal region of vastus lateralis, vastus intermedius and rectus femoris muscles. The electrodes were connected to the Digitimer

DS7AH via anode and cathode leads, as is illustrated in Figure 4.3.

The current (mA) was set to a very low value of 20 mA and a 30 Hz stimulation (i.e. 30 single pulses spread equally within 1 sec) was applied in order to familiarise the participant to the sensation of stimulation. The current was increased progressively and 30 Hz stimulation applied until the level was found that caused the knee extensors to contract at 30% of the MVC. The stimuli were controlled by a computer interface running custom written Matlab (Matlab, the Mathwork Inc., S Natik, MA, USA) and LabView programmes. The force of muscle contractions was digitised via a data-acquisition board (National Instruments Corporation, Texas, USA) and displayed using the LabView and Matlab programmes. Characteristics including peak amplitude, duration, rates of contraction and rates of relaxation could be calculated.

4.4.3.4.2.2 Participant's Position

Participants sat upright in the knee extension dynamometer with the hip and knees flexed at 90°. Seat adjustment for each participant depended on his lower limb length and girth. A secured strap was used over the hip area to restrict uncontrolled movement during knee extension contractions. The transducer measured forces (N) and was placed 2 cm above the lateral malleolus. Lever arm length was measured (in m) from the point of knee joint rotation down to the application of force against the transducer.

Figure 4. 3 **Position of participant and placement of electrodes**

4.4.3.4.2.3 Procedure of Measurement

Participants completed a warm-up that consisted of four consecutive sub-maximal isometric muscle contractions followed by a one minute period of rest. Following this, the maximum obtained force was measured during three MVC trails as participants were instructed to apply as much force as possible against the force transducer in three seconds, while receiving strong verbal encouragement from the researcher. The participant began a contraction following the examiner's verbal cue of "One, two, three, go!" This maximum effort was repeated twice more; the highest value being taken as MVC.

Once the accurate current was found, the forces of the quadriceps muscle were elicited by electrical stimulation at seven different frequencies: 1, 10, 15, 20, 30, 50, and 100 Hz, applied in a random order. Sixty second intervals were allowed between stimuli the tetani lasting for one full second. Peak force at each frequency, the degree of oscillation at 10 Hz and relaxation rate from the 50 and 20 Hz tetani were measured and used as an index of muscle contractile characteristics.

4.4.3.5 Exercise Step Test

It is well documented that eccentric exercise can result in muscle damage (Clarkson and Hubal, 2002). The intensity and length of exercise may bring a different level of damage to contractile and connective tissue components of skeletal muscle (McLeay et al., 2012). The step-up (concentric), step-down (eccentric) exercise protocol used in the Phase Two study has been validated several times and was the recommended protocol in a recent review (Allen, 2001). Eccentric action promotes greater tension to the contractile apparatus with greater accumulation of metabolites and structural damage in tissues (Pull, 2007). Participants performed the step exercise for 20 minutes using a 39 cm platform. The same leg was always used to step up, while the contralateral leg was used to step down. The leg selected to step up/down was chosen at random in the first visit. During the exercise, the leg being used concentrically feels fatigued because it is working at a considerably higher metabolic rate compared with the concentrically contracting limb. However, the following day and several days thereafter, it is the eccentrically-activated limb that experiences considerable muscle soreness. The well-trained athletes all wore an additional 16 kg weighted vest throughout the stepping exercise. An electronic metronome was used to provide timing of steps at a rate of two-seconds to step up and two-seconds to step down in a controlled manner.

4.4.3.6 Psychometric Measure

4.4.3.6.1 Pain

The amount of pain in the vastus lateralis was determined by using two methods. The first method was the visual analogue pain scale (VAS) (appendix 14), which consisted of a ten-centimetre line. On the left-hand side was a line representing no pain, and on the right-hand side was another line indicating the worst possible pain. VAS pain scores have been found to be valid and are a reliable standard for assessing pain intensity (Hjermstad et al., 2011), as well as important in clinical assessments of pain (Williamson and Hoggart, 2005). Participants in Phase Two were asked to indicate on the 10 cm horizontal line that corresponded to their intensity of pain while sitting, and prior to any functional testing (Waterfield and Sim, 1996). This was undertaken immediately after the recovery methods began, as well as at 24 hours and 48 hours in a rested state post damaging-exercise.

4.4.3.6.2 Pain Pressure Threshold Algometry (PPT)

Pain Pressure Threshold (PPT) is defined as the point at which a sensation of pressure converts to a sensation of pain (Andersen et al., 2006). PPT was measured by using a pressure algometer, which is a mechanical form of pain assessment. Moreover, PPT is considered to be a reliable technique for the assessment of mechanical pain according to the data in the literature (Binderup et al., 2010; Hong, 1999; Russell, 1999).

A Wagner algometer (model FDK/FDN, Wagner Instruments) was used in this study. The instrument has a 1-cm rubber footplate and a scale marked from 2 to 20 Kg. No calibration was required.

A line was drawn between the lateral border of the patella and the anterior iliac crest. PPTs were assessed in the vastus lateralis at three points along the line. Participants were seated in the chair with their knees at an angle of 90° with an algometer pressed perpendicularly onto the muscle at the marked spot. The participants were instructed to inform the researcher when the algometer's pressure changed to pain. The algometer's pressure reading was recorded, and this same pressure was used for that individual for the next two pressure recordings.

4.4.3.7 Ice Bath

A inflatable ice bath was used for the CWI. It was 72 cm high and could fill to a depth of 0.95 meters, as Figure 4.6 illustrates.

Figure 4. 4 **Inflatable Ice Bath**

The bath was filled with cold water to the level just below the sternum for each participant (to ensure that the iliac crest was fully submerged). Crushed ice was added until a temperature of 12-13°C was achieved. Meeusen and Lievens (1986) reported that 15°C could elicit a drop in intramuscular temperature. Thus, this water temperature was chosen because it appears as the most commonly used in previous CWI studies (Crow et al., 2007; Ingram et al., 2007; Vaile et al., 2008; Corbett et al., 2012). The water temperature was maintained at a constant temperature and was monitored by using a digital thermometer. Participants were immersed in the water bath in the seated position for 15 minutes.

4.4.3.8 Pilot Study

A pilot study is defined by (Leon et al., 2011) as "a small-scale test of the methods and procedures to be used on a larger scale". Six volunteers were invited to take part in a pilot study for this research in order to investigate the suitability of the assessment procedures. After the pilot study, the following criteria were taken into consideration when applying the procedures:

- The measurement of VO_2 was used in Phase One, but not in Phase Two because this form of measurement requires a longer period of time than the one-day test period, as each individual process takes 2 hours, besides the amount of time allocated to adjusting the device for each specific participant.
- The 16 kg weighted vest was added to the exercise session because the intensity of the stepping exercise alone was not anticipated to be of sufficient intensity to cause muscle damage in well-trained men.
- The duration of the session was determined so that individual appointments could be made for research participants.
- The optimum sample size was determined from the muscle electrical stimulation studies.

4.4.3.9 Crossover and Randomization

Phase Two of the study was a randomized crossover study so that each recruited participant would be randomly allocated to separately receive both active and control treatment with an interval of two weeks separating the conditions (Wellek and Blettner, 2012). Figure 4.7 illustrates the crossover method.

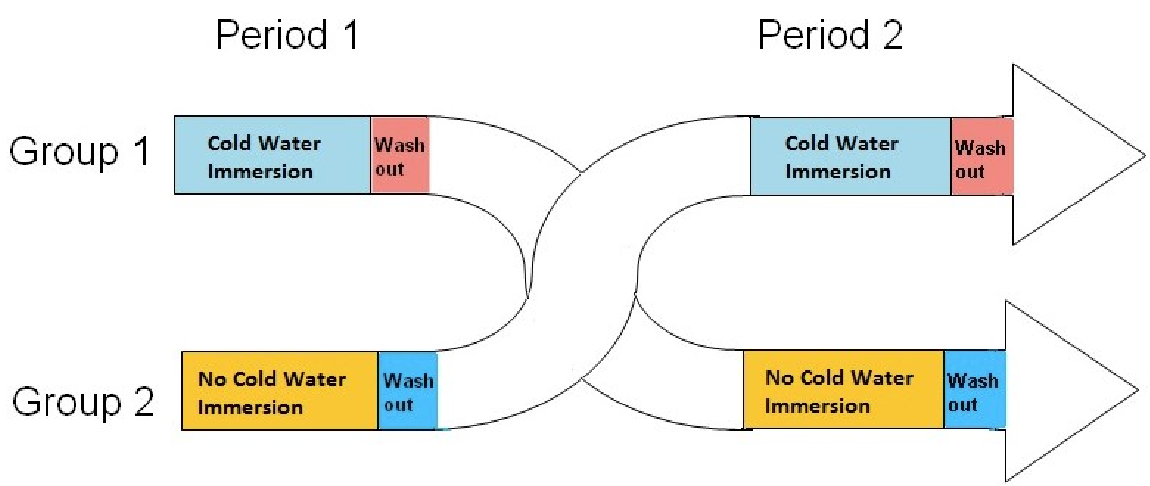


Figure 4. 5 **Crossover research design**

The disadvantage of using such a crossover design is that the effects of the first test period may have a direct impact on the outcome of the second period (Fitzpatrick & Downes, 2006). Hence, in order to ensure that the crossover design was used in an appropriate way the washout phase of two weeks was sufficiently long enough to eliminate any training effects. Relevant study have demonstrated that although muscle soreness and damage can frequently occur following varied routines of exercise, it commonly peak between 24 and 48 hours post-activity and effectively subsides within a 96 hour period (Glasgo et al., 2014). The repair mechanisms involve three stages: the destruction and inflammatory phase (1 to 3 days), the repair phase (3 to 4 weeks), and the remodeling phase (3 to 6 months) (Tidball, 2005). Furthermore, the leg that had not previously been used to perform the eccentric, downward stepping action was used in the second test so that any muscle damage symptoms that remained could be ruled out as a contributing factor and to reduce the ‘repeated bout effects’. Meanwhile, the advantage of using a crossover design is that it removes any variability between participants (Skinner, 2007).

Randomization was used to assign participants using an unbiased approach to the possible groups (Appel, 2006; Kang et al., 2008). Although randomization is usually associated with blinded trials (Saghaei, 2011), a simple randomization method was used in this study in the form of a singular coin flip to determine which leg would be the experimental eccentrically contracted leg in the first session.

4.4.3.10 Sample size calculation

Prior to data collection, the sample size was estimated in order to acquire the adequate sample size to achieve the research objectives (Jones et al., 2003). The method used to calculate the sample sizes in this study was based on the mean and standard deviation of a main dependent variable which was measured during the pilot study. (Suresh and Chandrashekara, 2012: 8) stated that, “the variance or standard division for sample size calculation is obtained either from previous studies or from a pilot study”.

Power calculations were conducted using G*Power. It was not possible to complete definitive a-priori power calculations because data were not available that showed the mean and standard deviation effect sizes for the type of exercise and cold water intervention used in this study. Therefore, calculations were based on data from a pilot study (*n* = 6) completed prior to the current study. The mean (67.2) and standard deviation (29.9) with an effect size of (0.44) values originating from the electrically-stimulated contraction of 15 Hz were used. A total sample size of 8 was estimated in order to achieve the power of 0.97 with an alpha of 0.05. Post-hoc power calculations based on the mean and standard deviation data from MVC before and 24 hrs after the exercise intervention from the current study (*n* =8) indicated 1-beta = 0.994, which suggested that the Phase Two study was suitably powered to show the main effect of intervention. The sample of phase one will be expanded in an attempt to reach to a wide base of data, as not enough information has been found in literature the.

4.4.3.11 Researcher Responsibility

The responsibility of the researcher began with writing the ethical approval documents. Prior to the recruitment stage for phase one, the researcher was responsible for ordering all necessary tools that were required for the study (ice bath, steps, needles, pads, thermometer, Pain Pressure Algometry device). Furthermore, the researcher was responsible for recording the operational procedures for the equipment used in the experiment and was responsible for the preparation of all required forms (information form, health status form, consent form). Once the equipment and procedure records were ready, the researcher was in charge of participants' recruitment, booking of appointments with participants and the sending of reminders until the test was terminated. On the day of the phase two test, the researcher was responsible for providing the two different investigators with written instructions and procedures for using the ice bath, and also to ensure that the amount of available ice was sufficient to cover the period of the test.

During the experiment, the researcher was responsible for all measurements for phase one, except for the blood sample; while for phase two, the researcher was in charge of obtaining muscle strength and pain measurements, besides distributing the questionnaire for day three. Once the data was collected from the study, the researcher was responsible for inputting all of the data into the statistical package for social sciences (SPSS).

4.4.3.12 Data analysis

The methodology applied in this study is a mixed methods design, with a combination of quantitative and qualitative research. The study is designed in three stages: the first and second are a quantitative analysis and the third is a mixed methods analysis. The specific data reduction for the quantitative study and the statistical analysis are presented throughout the experimental chapters (five-six). All data were analysed using the statistical software IBM SPSS Statistics 21 for Windows (Armonk, New York 10504-1722, USA). Each chapter outlines the appropriate tests used for the consideration of differences between the performances in participants. A level of significance was always accepted at $P \leq 0.05$ for all analysis. The mixed methods statistical analysis for Phase Three is outlined in detail within chapter seven.

Chapter 5

The Cardiorespiratory, Neuromuscular and Metabolic Responses to Cold Water Immersion

5.1 Introduction

Immersion of an unprotected body in cold water produces a rapid decrease in skin temperature, which subsequently evokes the initial responses to cold immersion; known by the generic name "cold shock" (Datta and Tipton, 2005). Cold induce non-shivering thermogenesis and it subsequent to produce heat. Non-shivering thermogenesis was originally defined as a cold-induced increase in heat production not associated with the muscle activity of shivering. This cold shock has an impact on the cardiorespiratory and metabolic response, as under cold circumstances, the body begins to shiver. This form of movement is the way that a non-exercising individual produces self-heat. Additionally, the body's response noticeably intensifies when the water submersion temperature is lowered. Nevertheless, the period preceding shivering has not been described in the literature review, as the focus of the study is structured around the actual "cold shock" period of shivering (Van Ooijen et al., 2005). Immersion in cold water affects an individual's physiological response at both rest and during exercise (Km et al., 2014). Even a slight drop in water temperature can produce a much greater cold stimulus, which then leads to major physiological changes (Lee et al., 1997). All the phsiological reactions from an individual to cold are instinctive to subsequently raise heat formation but limit heat loss (Weller et al., 1998). An individual's involuntary reaction is to decrease the superficial temperature, which is accompanied by modifications in circulation. Consequently, vasoconstriction of the arteries and veins, in the directly affected area, will be a direct result during limited exposures to cold (Van Ooijen et al., 2004). Vasoconstriction decreases the rate of skin circulation, and thus the temperature in the skin.

In fact, maximal vasoconstriction reduces heat conduction to about one third of its maximal value. It is the intensity of the cold, as well as the manner that the body is affected, which creates the amplitude of these mechanisms (Rintamäki et al., 2005).

Several other physiological actions have been attributed to the function of Cold Water Immersion (CWI), including an increase in cardiac output and blood pressure, but a reduction in muscle spasm, nerve conduction velocity (NCV), as well as in sensory and motor nerves (Tipton et al., 2000; Herrera et al., 2010). Furthermore, CWI has a controversial effect on muscle strength (force-generating capacity), as power and isokinetic strength appear to decline immediately following the application of various cryotherapeutic modalities (Patterson et al., 2008). Nonetheless, most research, which pertains to strength changes following cold exposure, has primarily been conducted on animals (Ranatunga and Wylie, 1983; Stephenson and Williams, 1981), while research on humans is largely informed by studies of isometric contractions of the upper extremities (Ranatunga et al., 1987; De Ruiter and Haan, 2000; Mattacola and Perrin., 1993; Bleakley et al., 2012).

The majority of relevant studies, which have dealt with human physiological responses to CWI, have used whole body, head-out immersion, limb-extremity immersion or athletic swimmers (De Ruiter and Haan, 2000; Foster and Sheel, 2005; Halson et al., 2008).

However, it has been demonstrated and reported that during rest in cold water the trunk is the major site of heat loss, and distal parts of the body are more insulative when compared to the trunk region (Lee et al., 1997). Nevertheless, there is minimal documented knowledge regarding the neuromuscular response of the muscles at rest without any physical activities to the CWI up to waist level. Therefore, this chapter's principal aim is to develop a comprehensive understanding of the neuromuscular response of an active individual to CWI up to waist level at rest.

5.2 Hypothesis

H0: There will be no changes in the physiological responses (HR, BP, VO_2) and muscle strength to the cold-water immersion (12-13°C) for fifteen minutes in a group of active healthy males.

H1: There will be changes in the physiological responses (HR, BP, VO_2) and muscle strength to the cold-water immersion (12-13°C) for fifteen minutes in a group of active healthy males.

5.3 Aims of the study

This study aims to determine:

- The physiological changes of heart rate, alongside blood pressure and oxygen consumption in an active individual after 15 minutes of CWI at 12°-13°C.
- The impact of 15 minutes of CWI at 12º-13°C on muscle strength during the

measurement periods.

5.4 Methods

5.4.1 Participants

Nine active volunteers participated in this study. Their demographics are presented in Chapter Four.

5.4.2 Study Design

The study design has been addressed in depth within Chapter Four, although Figure 5.1 below highlights the dynamics of the process over a two day period of measurement that distinguishes the physiological changes of nine active male participants through their HR, BP and oxygen consumption that were measured at four specific times during CWI.

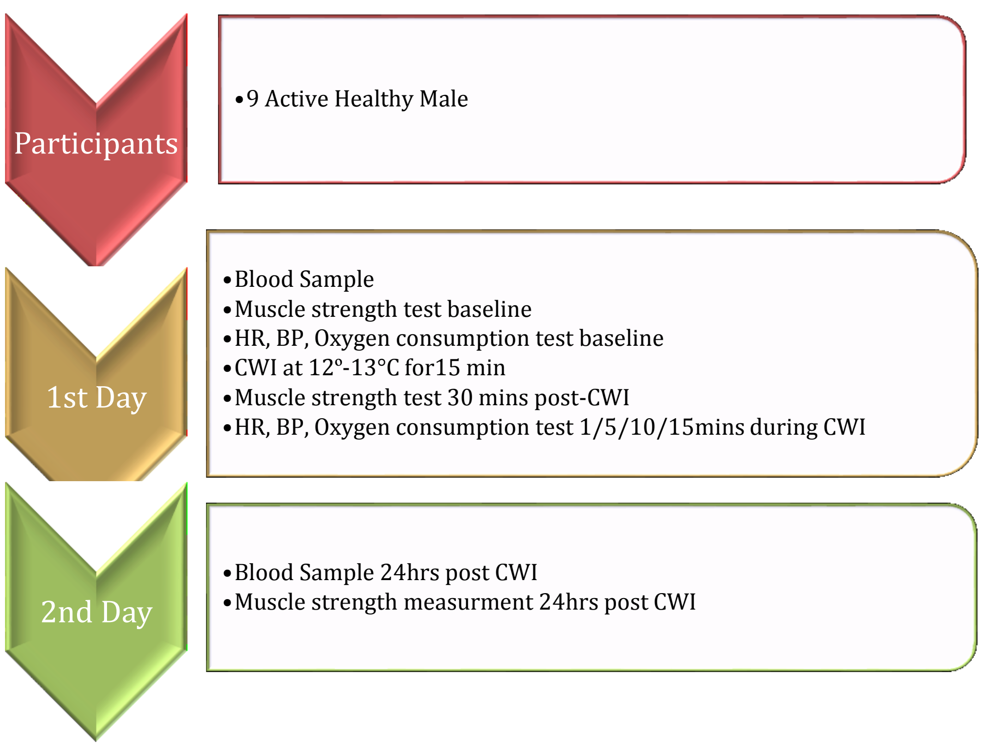


Figure 5. 1 Study Design

5.4.3 Heart Rate, Blood Pressure and VO_2 measure

The methods used to measure the heart rate and blood pressure during CWI through five time points (baseline, 1, 5, 10, 15 mins), as well as oxygen consumption are outlined in the methods chapter (Ch.4, section: 4.4.3.2).

5.4.4 Muscle strength Test

Muscle strength was determined using Cybex and ES measurement. A description of the methods that an individual was required to perform in order to measure the power of muscles is outlined in the method chapter (Ch.4, section: 4.4.3.4).

5.4.5 Blood Test

The methods for drawing blood samples and the hormones that measured are described in detail in the methods chapter (Ch.4, section: 4.4.3.1). The result of the blood will be addressed in chapter Eight.

5.4.6 Cold-water Immersion

A description of cold-water application is outlined in the method chapter (Ch.4, section: 4.4.3.7).

5.5 Data handling and statistical analysis

The statistical package of the social science (SPSS) Software for windows (v 21, Armonk, New York 10504-1722, USA) was used in this study for the statistical analysis.

Data are presented as mean and standard deviation and for all analyses the statistical significance was set as p-value ≤ 0.05 to understand the physiological changes (pre, immediate and 24-hour post-immersion). A series of One-way analyses of variance (ANOVA) were used to investigate of the statistical significant analyses. Furthermore, descriptive statistics, Repeated Measures ANOVA with post-hoc comparison between measurements were determined.

5.6 Results

5.6.1 Isometric maximal voluntary contraction (MVC)

A repeated measure ANOVA revealed that there was no significant change in MVC ($p=0.095$) through the measurement period, as shown in Figure 5.2.

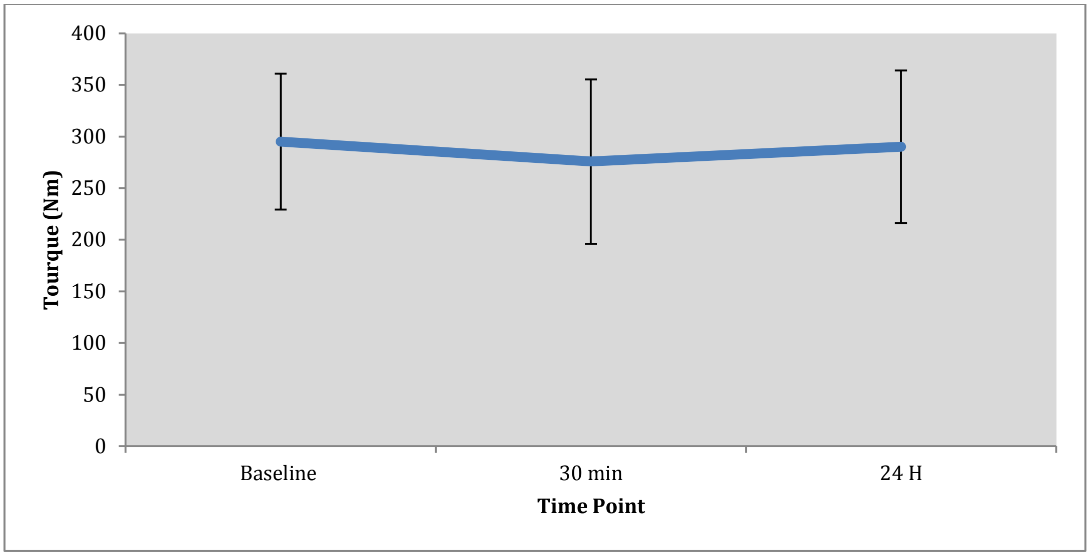


Figure 5.2 MVC at baseline, 30 min and 24 h post CWI. Data shown as mean +/- SEM. There were no significant changes to MVC between time points.

5.6.2 Effect of cold water on the torque-velocity relationship

Knee extensor muscle function was assessed at the different time points using a series of seven different velocities (60, 120, 180, 240, 300, 360, 420 deg/sec). There were very few changes over time, except for torque at 300°/s ($p=0.002$). It was significantly lower at 30 min post CWI ($p= 0.005$) and 24 hrs post CWI ($p= 0.033$) compared with baseline (Fig. 5.3).

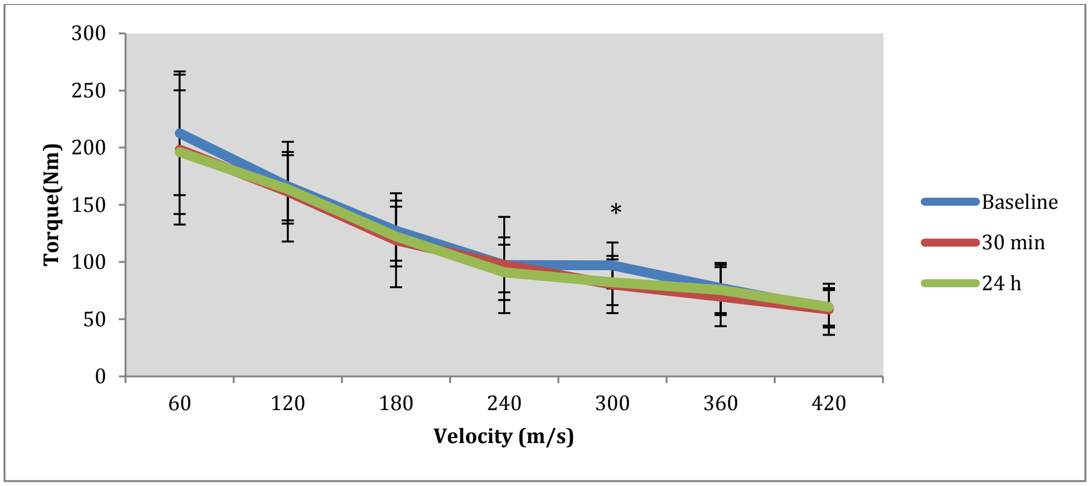


Figure 5. 3 Torque-velocity relationship of the knee extensors before and after CWI. (*) A significant Change in isometric force at (300°/s) over measurement time.

5.6.3 Effect of CWI on muscle contractile properties

Contractile characteristics were assessed at the three different time points (baseline, post and 24 h post) using a series of 7 different frequencies (1, 10, 15, 20, 30, 50 and 100 Hz) of electrically stimulated contractions (Fig. 5.4). There were no significant changes in force at any frequency of stimulation across the three time points.

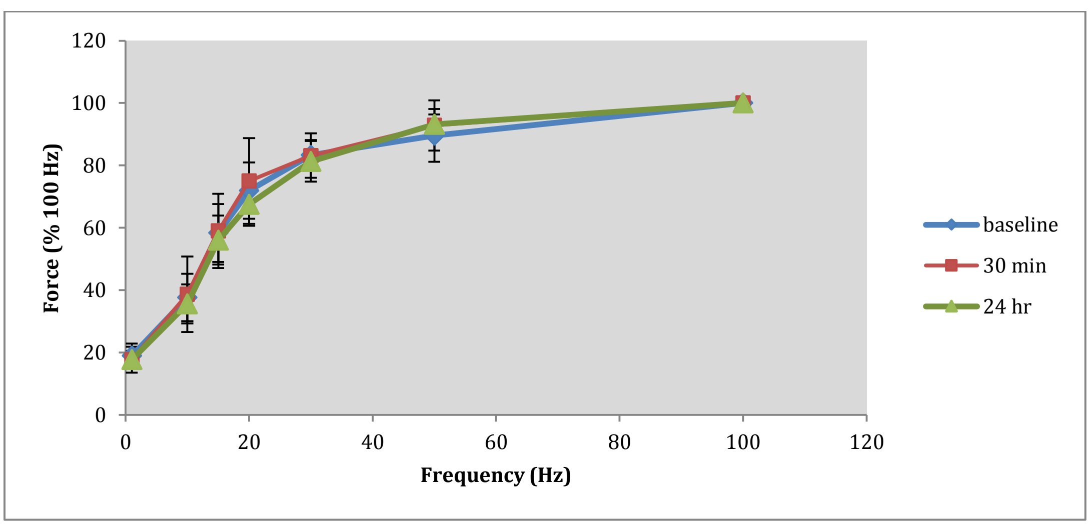


Figure 5. 11 Force-frequency relationship.

5.6.4 Heart Rate

Heart rate was recorded at five time points to monitor the response to cold-water immersion (baseline, 1minute, 5 minutes, 10 minutes and 15 minutes; Table 5.5), but it did not change significantly over time (p=0.531). While HR in most participants tended to increase within the first 60 sec of CWI, there were two in whom HR decreased considerably and it remained low for the period of the CWI.

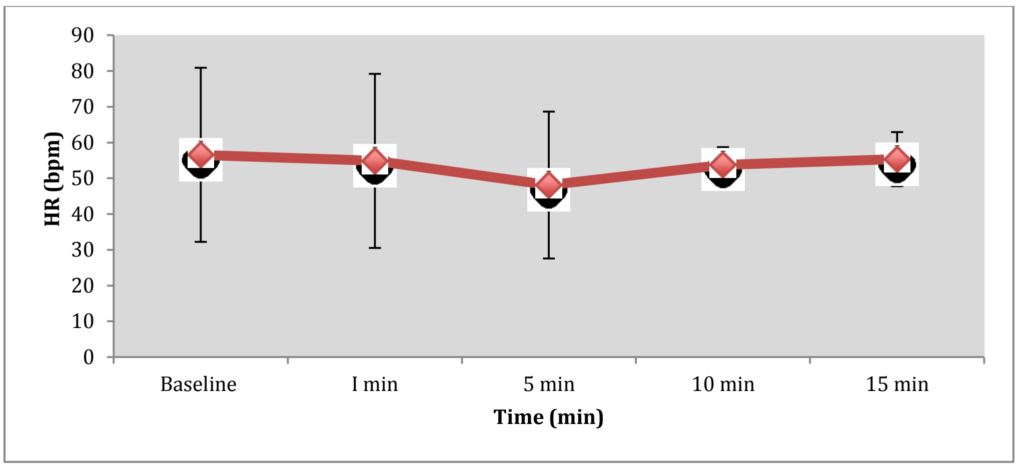


Figure 5. 12 Heart Rate response during cold-water immersion to the waist level thorough different five-time measurement.

5.6.5 Blood pressure

5.6.5.1 Systolic and Diastolic

Systolic blood pressure showed a significant response to CWI (p=0.014). From baseline, it decreased within the first five min (p=0.020) and then returned to resting levels by 10 min (Fig 5.6). The pattern of response for diastolic BP was similar to that of systolic, but the changes were not significant (p=0.117; Fig 5.7).

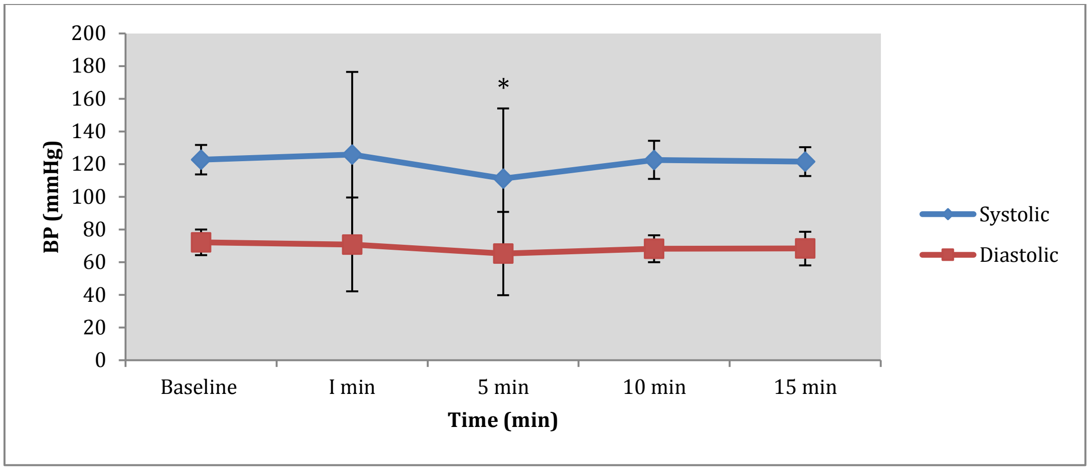


Figure 5.7 effect of cold water on blood pressure (diastolic and systolic) at five different periods of time. (*) A significant change in the systolic level at 5 min during cold-water immersion.

5.6.6 Effect of cold water immersion on breathing rate and metabolism

Oxygen consumption was assessed during the period of CWI. Results showed that VO_2 did not significantly change during the first minute of immersion. However, there was a trend ($p= 0.079$) of increase that remained up to the second minute ($p= 0.092$), but which then returned to the baseline after three minutes ($p=0.741$). Furthermore, there were no significant changes in the levels of minute ventilation (VE) ($p=0.207$), carbon dioxide production (VCO_2) ($p=0.061$) and respiratory exchange ratio (RER) ($p=0.158$) during measurement time.

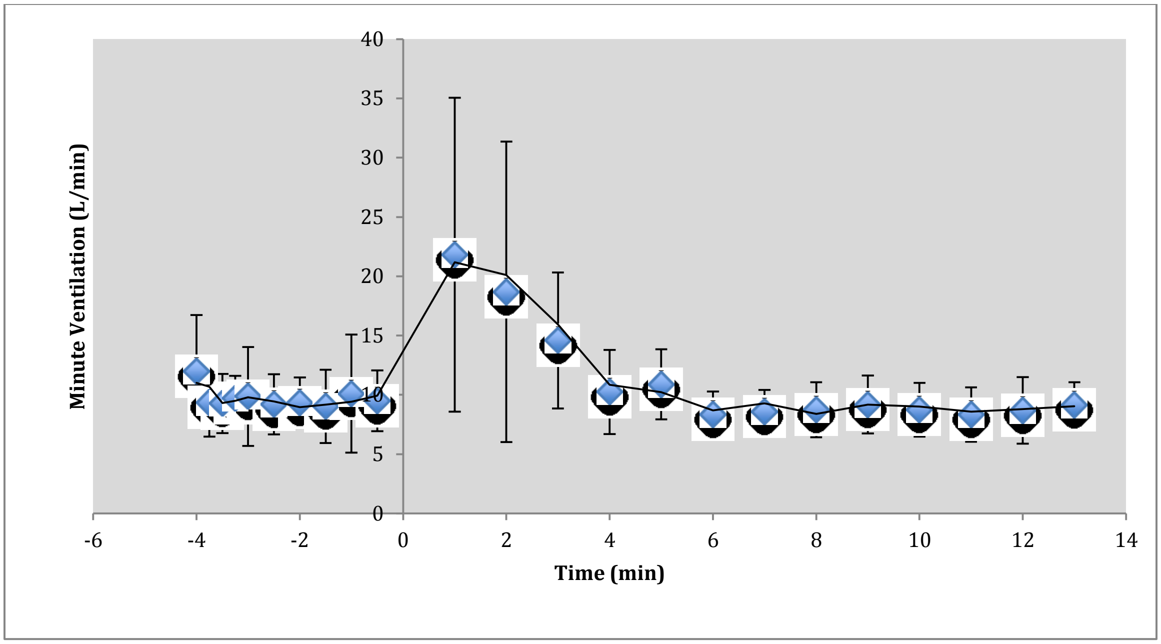


Figure 5.8 Minute ventilation during cold-water immersion. Values at time 0 min measured during 5 min baseline period (seated in chair)

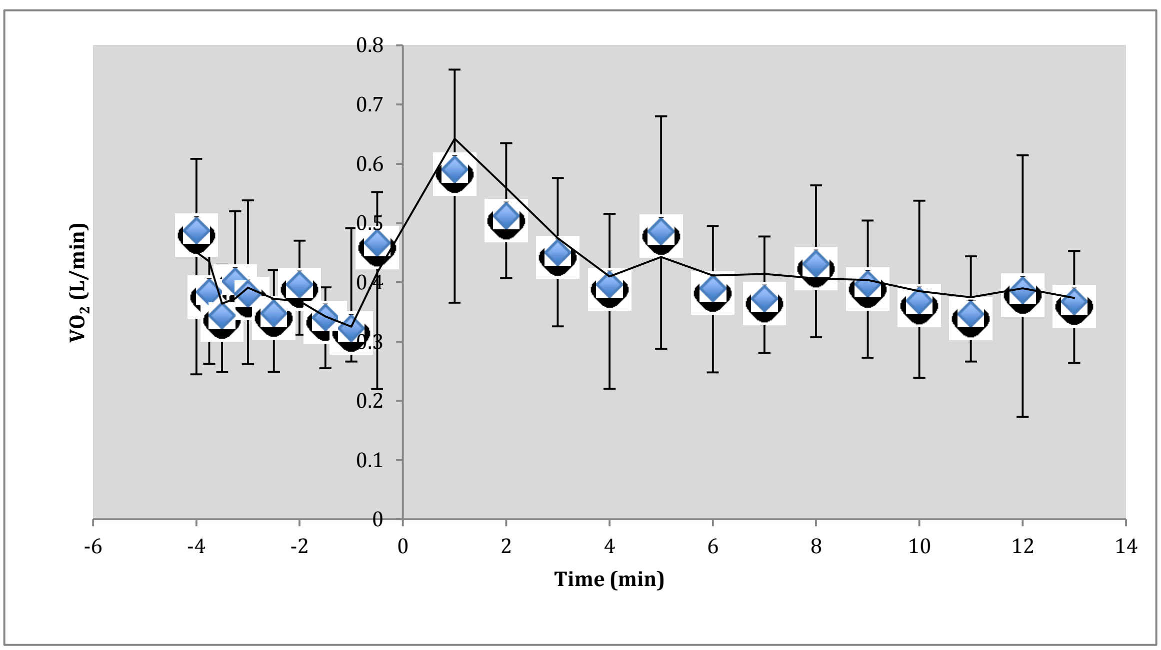


Figure 5.9 Rate of oxygen consumption (VO_2) during cold-water immersion. Values at time 0 min measured during 5 min baseline period (seated in chair)

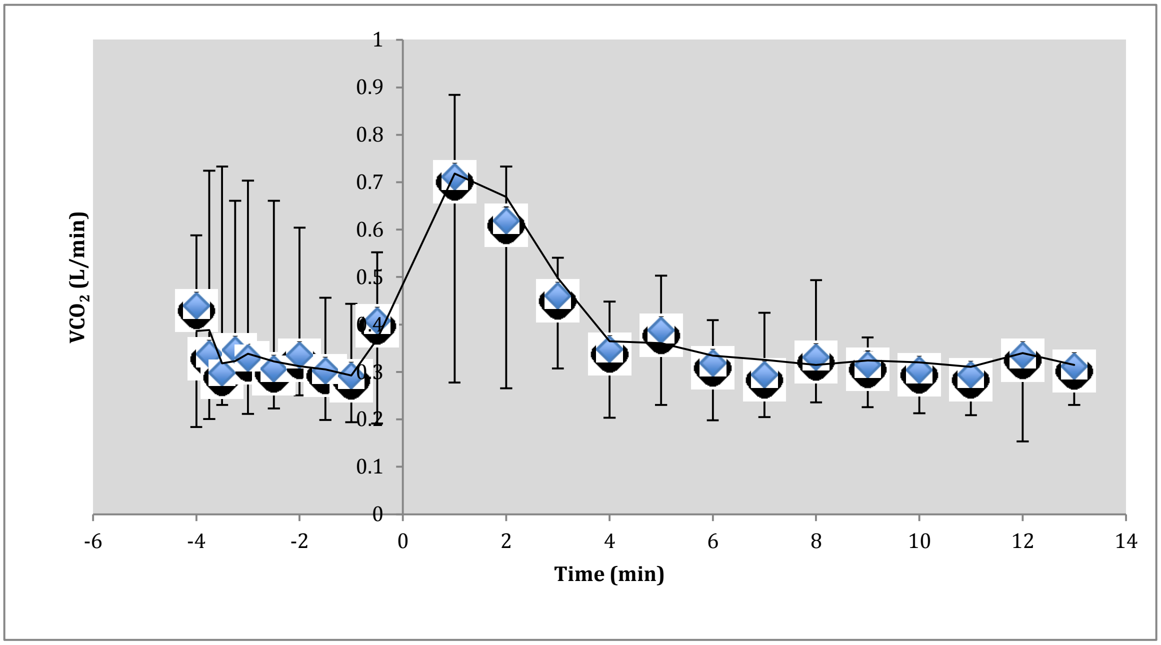


Figure 5.10 Carbon dioxide productions (VCO₂) during cold-water immersion. Values at time 0 min measured during 5 min baseline period (seated in chair)

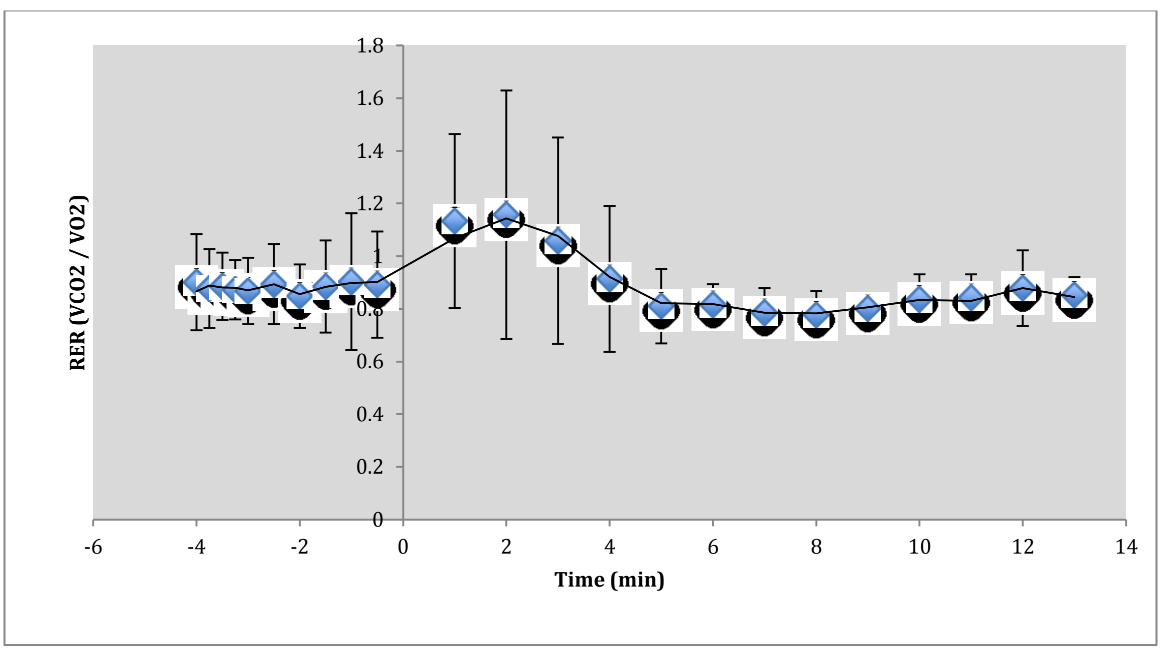


Figure 5.11 Respiratory exchange ratio (RER= VCO_2/ VO_2) during cold-water immersion. Values at time 0 min measured during 5 min baseline period (seated in chair).

5.7 Discussion

The experiment was performed to describe the acute cardiorespiratory response to cold-water immersion, and the subsequent neuromuscular consequences 30 minutes and 24 hours after. The results showed no significant effect on neuromuscular function, but a clear tendency towards altered cardiorespiratory function immediately upon exposure to CWI that was normalized within 3-5 min, but due to large variability between people in the magnitude, and even in the direction of these responses, the effects were not significant.

Immersion in cold water may exert an influence on the human body by the activation of the sympathetic nervous system and endocrine function (Sramek et al., 2000). However, the physiological response to CWI depends upon the level of immersion and the temperature of the water (Lee et al., 1997). In the present study, participants did not experience any alteration in blood pressure, except for a slight decrease in systolic BP at 5 min after initial exposure. This could be because the participants were immersed only to waist deep, and greater effects may follow whole-body immersion or exposure to colder water (Sendowski et al., 1997). Several previous studies reported increased blood pressure in response to CWI (Golden et al., 1997; Foster and Sheel, 2005; Janský et al., 2006). However, these differences may have been the result of whole-body exposure (Golden et al., 1997), or due to the fact that participants were exposed to multiple CWI periods (Jansky et al., 2006). Others reported no changes in arterial blood pressure after immersion in water of 34°C at various levels, including up to the knee or xiphoid process, fourth intercostal space, and sternoclavicular notch (Gabrielsen et al., 1993; Boussuges,2006). Conversely, Korhonen (2006), reported that wrist and foot immersion for just 1 minute in iced water (2°C) caused an increase in systolic and diastolic blood pressure. Variations between people in the blood pressure responses could be due to individual differences in physiological vasomotor reactions (van Ooijen et al., 2005).

Similar to blood pressure, heart rate in this study did not change significantly following CWI, which is consistent with other reports (Janský et al., 1996; Park et al., 1999; Strejcová and Konopková, 2012). Nonetheless, a few studies have reported an increase in HR after CWI (Schipke and Pelzer, 2001; Janský et al., 2006). The marked difference between the previous studies and the current research is most likely due to methodological variations. Korhonen (2006) stated that an increase in the sympathetic nervous system occurring upon exposure to 2°C heightens heart rate. Hence, the insignificant change in HR within the current study may be explained by the water temperature of 12-13°C being insufficient to stimulate the sympathetic nervous system. However, previous research invariantly reported that 14 minute of immersion on a degree of 15°C this level of coldness would be adequate in eliciting a drop in the intramuscular temperature by 10°C and potentially have a beneficial effect, which is the reason for the chosen temperature that was employed in this study (Meeusen and Lievens., 1986). Another explanation for the lack of change in HR in the present study could be the depth of exposure. A study conducted by Tipton (1989), showed that HR increases through whole-body (except for the head) immersion after 2 minutes in water at 5, 10 and 15°C. Sensory cold receptors are not evenly distributed across the skin (Gagge et al., 1969) and cold sensation is also a subjective feeling, which may account for differences between studies.

The increase in standard error of the mean VO_2 and VCO_2 by around two-fold in the 2-3 minutes following initial CWI exposure indicates some physiological breathing and/or metabolic responses. However, the response was variable between individuals, with some increasing and others decreasing VO_2 and VCO_2, which is likely due to variable breathing responses (Fig 5.8), so the mean VO_2 and VCO_2 responses were not significantly changed following CWI exposure. These results are consistent with previous research that reported variability between people in breathing responses (Tipton et al., 1991; Datta and Tipton, 2006; Bleakley and Davison, 2009) linked to a cold-induced reflex increase in VE described by Tipton (1992).

The results of the current study have demonstrated no differences in KE MVC from pre-, 30 min post-, and 24h post- CWI. Some previous studies did show that cooling has an effect on maximal force, velocity, power and endurance (Holewijn and Heus, 1992; Oksa et al., 2000), although Bergh and Ekblom (1979) also reported no change in MVC after warm water or cold water immersion. In contrast, other studies have shown that dorsiflexor MVC strength declines after 20 minute of cooling at 10°C (Oksa et al., 2000; Oksa, 2002; Halder et al., 2014). In the present study, the torque-velocity relationship was also assessed and a decline was evident at 300°/s post CWI compared with baseline values. The fact that other velocities of contraction were unaffected and that the baseline results actually show a high value for torque at 300°/s relative to the overall trend (decrease in torque as velocity increases), and that (Kimura et al., 1997) found no change in torque at set contraction velocities, suggest this result should be treated with caution. However, others reported a decrease in peak torque post-CWI (Holewijn and Heus, 1992; Mattacola and Perrin, 1993; Mäkinen, 2007; Strejcová and Konopková, 2012)Rutkove (2009) and any differences between results may be accounted for by the motivation of participants. This factor can be overcome by superimposing electrical stimulation over the muscle. In the present study, stimulation at frequencies ranging from 1 to 100 Hz did not change the force produced (Fig 5.11) after CWI. However, these results contradicted with the results of another study that observed changes in the contractile properties of the triceps surae muscle following cooling (Davies et al., 1982). The differences in the results between the current and previous studies may be due to the type and duration of the cold exposure (cold water or air, whole body or extremities). Any decline in force after CWI may be related to reduced release of calcium from the sarcoplasmic reticulum, and thus fewer cross-bridges in peak tension (Holewijn and Heus, 1992)(Westerblad et al., 2000; Place et al., 2010). The contractile force of a muscle is significantly reduced when its temperature falls below 27°C due to reduced enzyme activity and increased co-activation of antagonist muscles (Oksa et al., 1995; Tipton and Bradford, 2014) and altered nerve conduction velocity (Rutkove, 2001; Juha Oksa, 2002; Dewhurst et al., 2005). Any substantial decrease in muscle temperature is therefore likely to impair physical performance (Mäkinen, 2007).

5.8 Limitations of the study

The main limitation of the present study is that skin and muscle temperatures were not measured, so it is not possible to know how sensory neurons sensitive to cold were activated, nor was it possible to know the intramuscular temperatures. To gain a clearer understanding into the benefit of CWI on actual muscle performance, it would be necessary to ascertain intramuscular temperature measurements, as previous studies have highlighted. Furthermore, there was up to a 1-hour delay after CWI before testing muscle strength in some participants, which may have caused the temperature of the muscle to return to normal, but these results nevertheless represent longer-lasting effects on muscle.

5.9 Conclusion

The objective of the current study was to acquire a clear understanding of the physiological response of CWI at 12-13°C for 15 minutes in active healthy participants. The findings suggest that following CWI, no clear effects upon cardiovascular and muscle strength were evident.

Chapter 6. The Effect of cold water immersion on neuromuscular recovery in elite rugby players: a randomised, controlled, cross-over trial

6.1 Background

Elite athletes must maintain a consistently high level of performance and aside from factors that affect motivation, physical injury or muscular damage such as delayed-onset muscle soreness (DOMS) can lead to decrements in performance,(Bailey et al., 2007; White et al., 2014). It is therefore important that athletes receive adequate recovery following intense physical exertion (Crowe et al., 2007).

Post-exercise recovery, which refers to a period of psychological and physical regeneration (Vaile et al., 2008), is an essential part of any training program, as it may allow athletes to overcome the demands of training and competition (Higgins et al., 2011). Hence, it is commonplace for athletes to utilize post-exercise strategies or practices in order to speed up their recovery (Vaile et al., 2008).

Recently, CWI has been considered as one of the most popular post-exercise interventions (Bleakley and Davison, 2009; Corbett et al., 2012). This strategy is utilised particularly with high-level athletes, in an effort to reduce muscle soreness and to hasten a return to optimal performance capabilities (Sellwood et al., 2007; Ingram et al., 2009). As a matter of fact, it has been speculated that the intervention of CWI often causes peripheral vasoconstriction, as well as reducing inflammation. As a consequence, this process creates a reduction in the athlete's pain and provides greater muscle recovery post-exercise (Santos et al., 2012). Accordingly, the results from the previous chapter investigated the response of the cardiovascular and neuromuscular systems post-CWI following no physical activity in attempt to observe whether there was a decline or enhancement in strength after CWI when no exercise had been undertaken.

The scientific basis for CWI as a recovery intervention remains equivocal. There is inconsistent evidence concerning the effects of CWI on post-exercise recovery in team-sports (White and Wells, 2013). In the current study, physiological and psychological variables were examined using an RCT study design comparing CWI recovery against passive recovery.

6.2 Hypothesis

H0: CWI will not decrease the level of soreness, or increase strength and inflammatory markers compared to the control group in rugby athletes following muscle damage.

H1: CWI will decrease the level of soreness, and increase strength and inflammatory markers compared to the control group in rugby athletes following muscle damage

6.3 Aims

6.3.1 Primary aim: The primary aims of the RCT Study were:

- To compare the effect of CWI on muscle strength measured using isokinetic dynamometer and ES between those receiving CWI and those who recovered passively post-EMID.
- To investigate the effect of CWI on the biochemical markers (e.g. cortisol and cytokines) post-EMID.

6.3.2 Secondary aims: The secondary aims of the RCT Study was:

- To compare the effects of CWI on muscle soreness measured by VAS and PPT in two conditions after intense eccentric stepping exercise (stepping down): those who received the treatment and the control condition who did not.

6.4 Methods

6.4.1 Participants

Eight Welsh Rugby players volunteered to participate in this study. Their demographics are present in the method chapter [chapter Four].

6.4.2 Study Design

A randomized crossover design was used with the exact replicated procedures for 8 elite rugby players following a two-week interval after the initial measurements were taken with the same group. The study design has been addressed in depth in the method chapter, although Figure 6.1 highlights the dynamics of the structure.

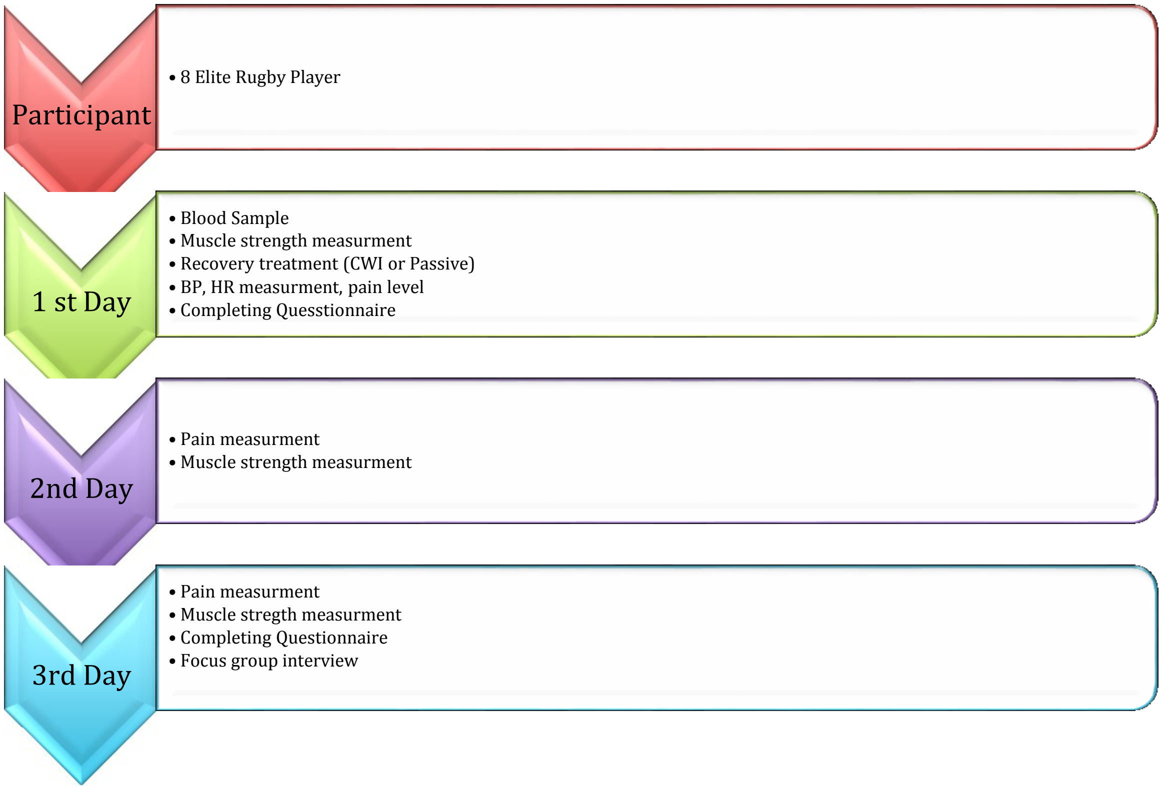


Figure 6.1 Study Design Phase Two

6.4.3 Psychometric Measures

Visual Analogue Scale and pain pressure threshold were used in this study. The details of the methods used to determine muscle soreness and pain threshold are outlined in the method chapter [chapter four].

6.4.4 Heart Rate and Blood measure

The methods used to measure the heart rate and blood pressure during CWI through five time points (baseline, 1, 5, 10, 15 mins) are outlined in the methods chapter (Ch.4, section: 4.4.3.2).

6.4.5 Muscle strength

Muscle strength was determined using Cybex and ES measurement. A description of the methods that an athlete is required to perform in order to measure the strength and power of muscles is outlined in the method chapter (Ch.4, section: 4.4.3.4).

6.4.6 Recovery Interventions

A description of post-training recovery intervention, as carried out by athletes, is fully outlined in the method (Ch.4, section 4.4.3.7), which highlights the process of either CWI or passive seated rest upon a chair for a period 15 minutes.

6.5 Data Analysis and statistic consideration

The statistical package of the social science (SPSS) for windows (v 21, Armonk, New York 10504-1722, USA) was used for the statistical analysis. Data are presented as mean and standard deviation, and for all remits of analysis, statistical significance was set as $\alpha = 0.05$ (at the confidence level of 95%). One-way analysis of variance (ANOVA) was used to compare pre and post psychometric and physical performance data between cold water and non-cold water groups. Subsequently, these results were compared by a two-factor variance analysis of time and group. Descriptive statistics, which lead to repeated measures Analysis of Variance (ANOVA) was used, in line with post-hoc comparison between measurements. Data were normally distributed and are presented as mean and standard deviation.

6.6 Results

6.6.1 Effect of CWI on Blood Pressure (BP)

The results of repeated measures ANOVA showed no significant increase in systolic ($p=0.119$) or diastolic blood pressure ($p=0.107$) over the course of the measurement time during immersion in the cold-water group.

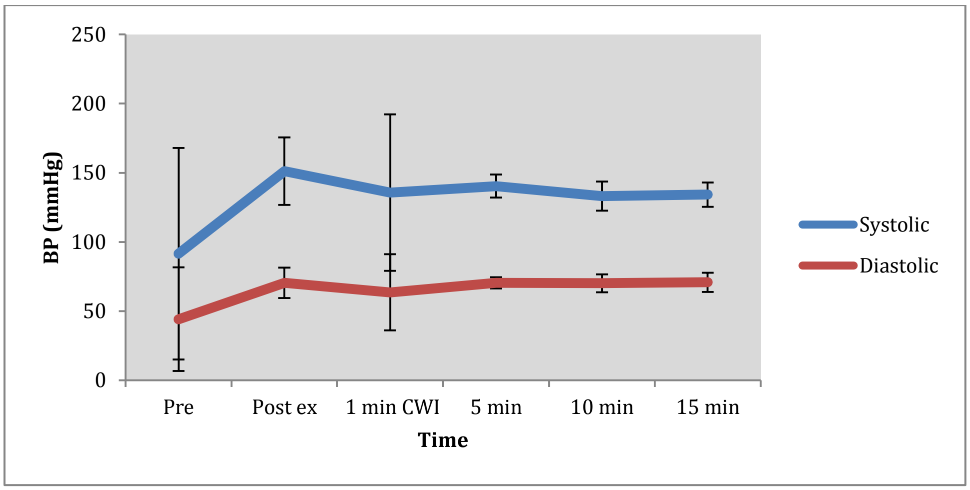


Figure 6.2 Systolic and diastolic blood pressure measurement throughout CWI.

6.6.2 Effect of CWI on heart rate (HR)

A repeated measure ANOVA showed a significant increase in heart rate over the course of the four-measurement times during immersion in the cold-water group ($p<0.001$). Heart rate increased significantly in the first minute of immersion ($p=0.031$), then started to decline within 5 minutes post immersion ($p=0.544$) and returned to the baseline 10 minutes after ($p= 0.995$).

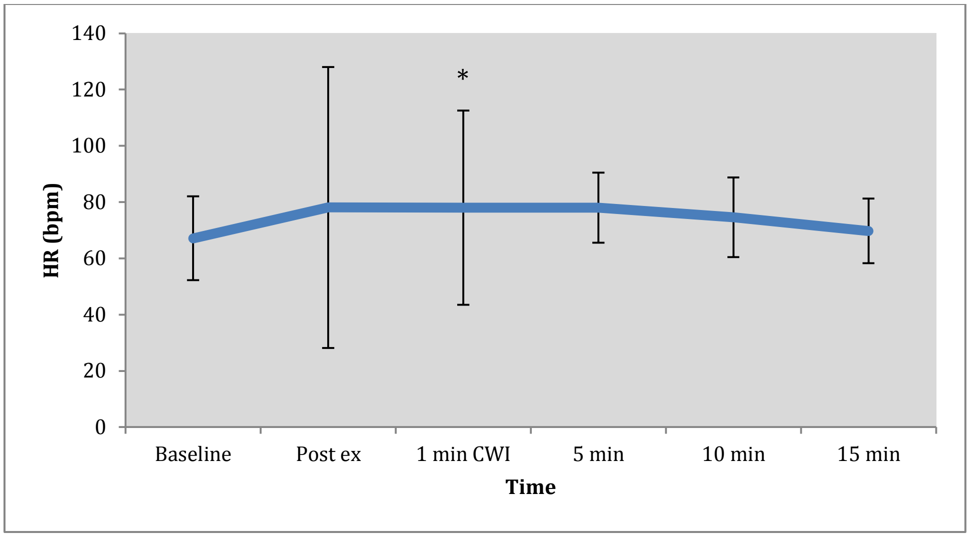


Figure 6.3 Heart rate measurement throughout CWI. (*) significant increase at first minute of immersion.

6.6.3 Effect of Recovery on muscle soreness

The visual analogue scale was completed immediately post muscle damaging exercise, as well as 24hr and 48hr post-intervention. A repeated Measures ANOVA (3 time vs 2 groups) showed no significant increase in self-reported muscle pain over the follow-up period ($p=0.299$) and no differences between treatment conditions ($p=0.109$).

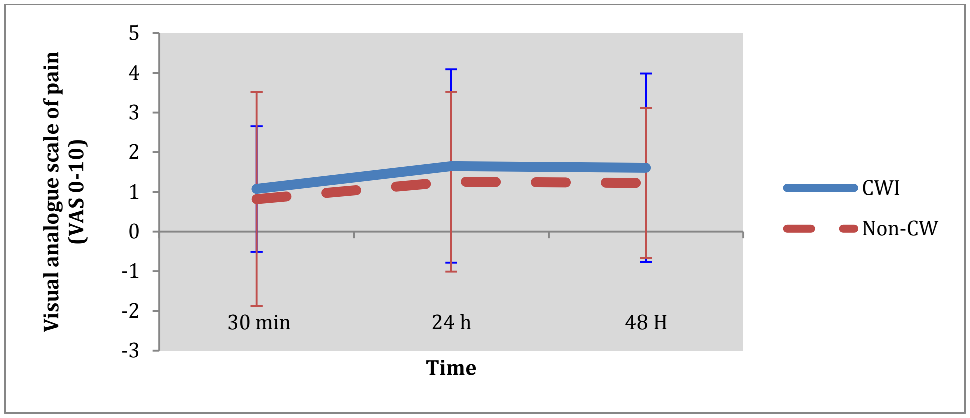


Figure 6.4 Average self-reported soreness for CWI& Non-cold. No significant differences between groups at any point.

6.6.4 Pressure Pain Threshold (PPT)

PPT was measured in 3 locations of vastus lateralis, which represented proximal, mid, and distal regions. A repeated measure ANOVA showed that PPT was significantly higher at 30 min in the CWI group at the proximal location (p=0.006) (Fig. 6.3), with no difference between treatment conditions in the location of highest pain (p=0.922).

In the distal location, PPT did not change at any time point after the cold-water treatment (p=0.275) or in the control condition (p=0.197). In the mid-muscle location, PPT did not change significantly over time in the cold (p=0.159) nor in the non-cold group (p=0.422). In the proximal muscle location, PPT did not change significantly over time in the CWI group (p=0.275), but decreased significantly in the non-cold group (p=0.018). The PPT at the proximal location was significantly lower in the non-cold group compared with the cold group (p=0.006), indicating greater levels of muscle tenderness during the pressure test in the non-cold group.

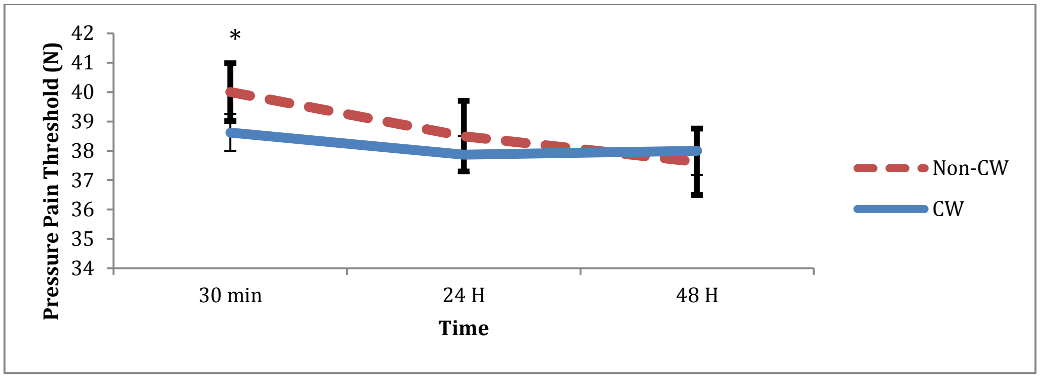


Figure 6.5 Pressure pain threshold (PPT) between (CWI & Non-cold). (*) significant differences between groups (p=0.006) at proximal location.

6.6.5 Effect of Recovery on isometric maximal voluntary contraction (MVC)

A repeated measures ANOVA revealed that KE MVC significantly changed over the course of the four-measurement time points in the cold-water condition ($p= 0.004$) and the non-cold water condition ($p=0.002$). KE MVC was lower at 15 minutes post-intervention (approx. 25 min after exercise) in both the cold ($p=0.003$) and non-cold ($p=0.016$) water conditions. By 24hr post-intervention, muscle strength had recovered in both cold- and non-cold water conditions (compared with baseline, $p=0.112$ in non-cold; and $p=1.000$ in cold). No significant difference was recorded between groups at any measurement time point (all $p>0.005$).

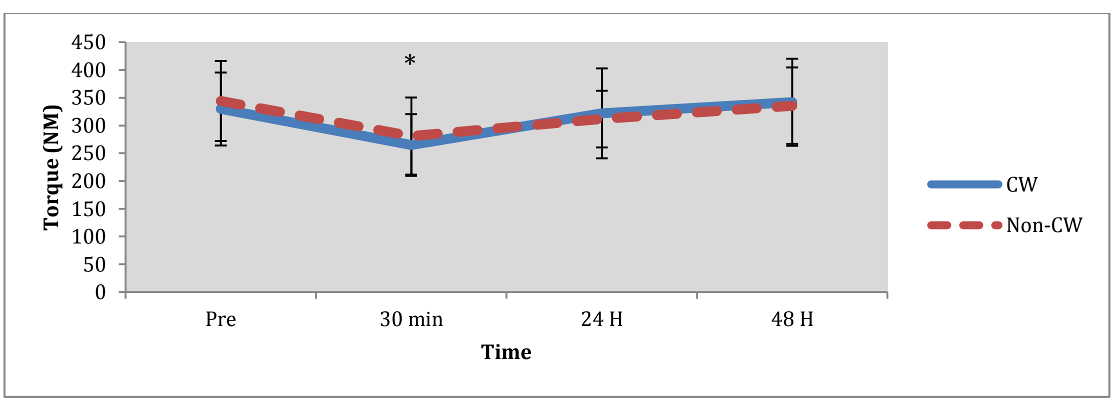


Figure 6.6 Change in the maximal voluntary contraction torque. Change in the KE MVC over time. Both cold-water and non-cold water conditions lost force at 30 min-post muscle-damaging exercise, but strength had recovered by the following day. (*) Indicates the decrement of the force for CW (P= 0.004) & Non-cold (P= 0.002). However, there was no difference between treatment conditions.

6.6.6 Effect of recovery on muscle contractile properties

Contractile characteristics were assessed at the different time points by the utilization of a series of 7 different frequencies of electrically stimulated contractions.

In the cold-water condition, Repeated Measures ANOVA showed that there was a significant effect on recovery time (P<0.000) through 1, 10 and 15 Hz stimulation. For 1 Hz, twitch torque was decreased 15 minutes after cold-water immersion (P=0.008) and 24hr later (P=0.056), but the muscle had recovered by 48hr (P=0.998). For 10 Hz, twitch torque was decreased 15 minutes after cold-water immersion (P=0.001), although the muscle had recovered by 24hr (P=0.173) and 48hr (P= .995). For 15 Hz, twitch torque was again decreased 15 minutes after cold-water immersion (P=0.005) and had recovered once more by 24hr (P=0.067) and 48hr (P=0.995). At 20 Hz, the effect of time did not quite reach the level of significance in the cold water group (P=0.053), but torque amplitude was significantly lower after 15 minutes (P=0.995) and 24hr (P=0.036) cold-water immersion compared with 48hr later. The higher frequency stimulations showed the same overall trends as the lower frequency of reduced torque amplitude immediately after CWI, with steady progressive recovery from 24 to 48 hrs. However, this did not reach statistical significance for 30 Hz (P=0.075) or 50 Hz (P=0.112).

In the non-cold water condition, Repeated Measures ANOVA showed that there was a significant effect on stimulated torque through 1, 10, 15, 20 and 30 Hz stimulation (all $P<0.05$). For 1 Hz, twitch torque was decreased 15 minutes after exercise ($P=0.002$) and had recovered by 24hr ($P=0.740$) and 48hr ($P=0.337$). For 10 Hz, twitch torque was decreased 15 minutes after intervention ($P=0.0005$) and had recovered by 24hr ($P=0.115$) and 48hr ($P=1.000$). For 15 Hz, twitch torque was decreased 15 minutes after intervention ($P=0.005$) and had recovered by 24hr ($P=0.156$) and 48hr ($P=0.995$). At 20 Hz, the torque amplitude was reduced 15 minutes after intervention ($P=0.002$), but had recovered at 24hr ($P=0.358$) and 48hr ($P=0.995$). At 30 Hz, torque was decreased 15 minutes after intervention ($P=0.054$) and it remained lower at 24hr ($P=0.041$), but recovered by 48hr ($P=0.166$). At 50 Hz, there were no significant effects on recovery ($P=0.112$).

A Two-way ANOVA found that there were no significant group * time interactions for 1 Hz ($P=0.625$) or 10 Hz (0.404) or 20 Hz ($P=0.092$) or 30 Hz ($P=0.433$) or 50 Hz ($P=0.531$). Although, it was noted that the non-cold water condition tended to lose a greater amount of torque at all stimulation frequencies and this reached the level of significance at 15 Hz ($p=0.039$).

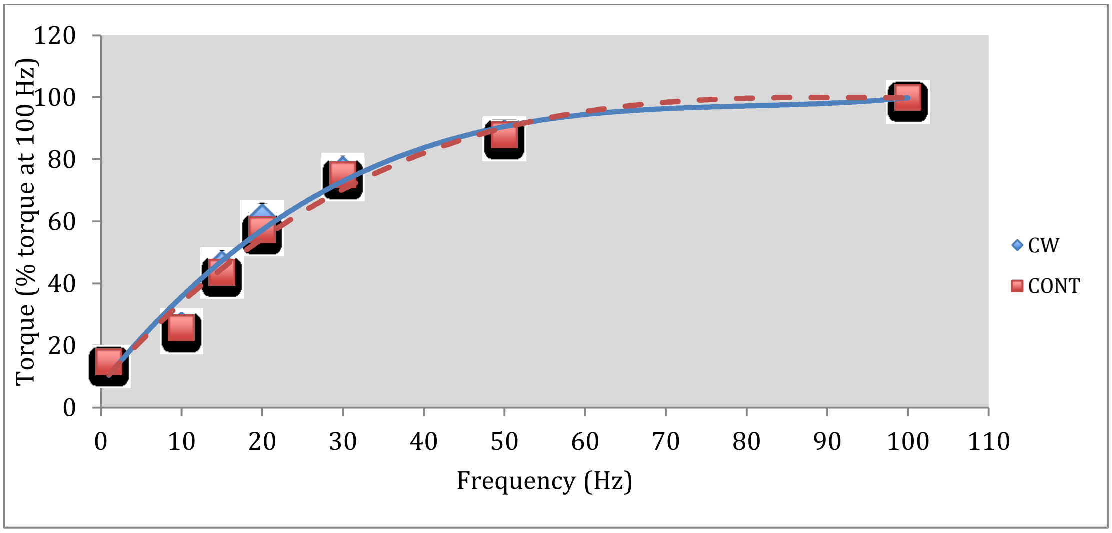


Figure 6.7 The torque-frequency relationship. Baseline for cold and Non-cold water groups.

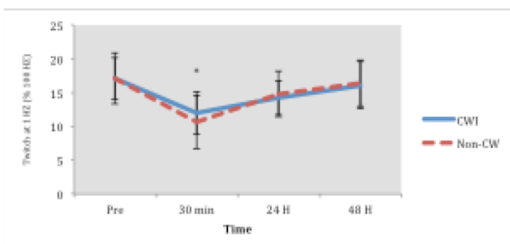


Figure 6.8 Changes in isometric twitch torque at 1 Hz. Changes in isometric twitch torque after muscle damaging exercise: (*) a significantly different from baseline (CWI p =0.008, Non-CW p=0.002).

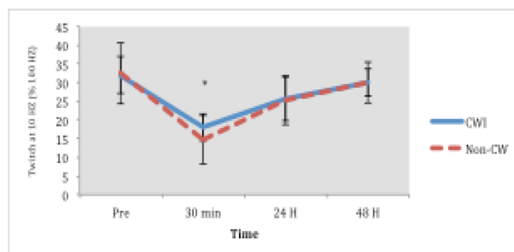


Figure 6.9 Changes in isometric twitch torque at 10 hz. Changes in isometric twitch torque after muscle damaging exercise: (*) a significant difference 30 min post CWI (CWI p =0.001, Non-CW p=0.005).

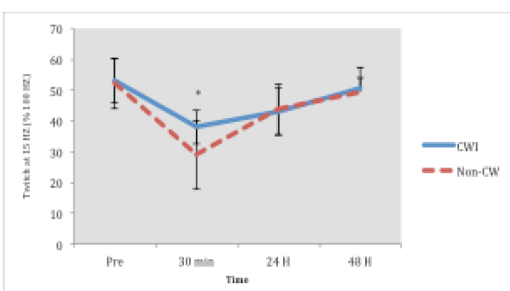


Figure 6.10 Changes in isometric twitch torque at 15 hz. Changes in isometric twitch torque after muscle damaging exercise: (*) a significant difference 30 min post CWI (CWI p =0.005, Non-CW p=0.005).

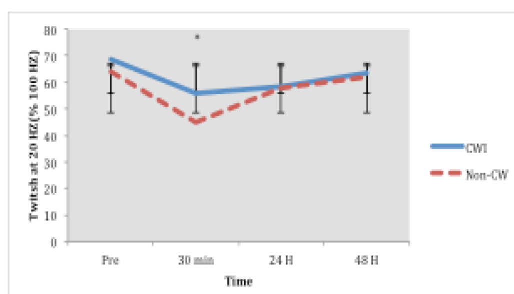


Figure 6.11 Changes in isometric twitch torque at 20 hz. Changes in isometric twitch torque muscle damaging exercise: (*) a significant difference 30 min post CWI (CWI p =0.053, Non-CW p=0.005).

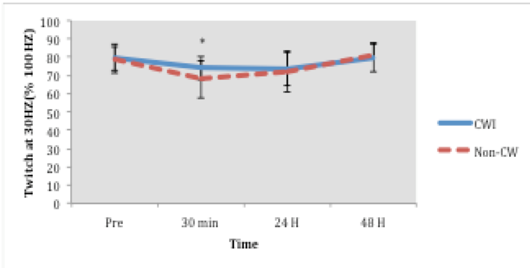


Figure 6.12 Changes in isometric twitch torque at 30 hz. Changes in isometric twitch torque after muscle damaging exercise: (*) a significant difference 30 min post CWI for Non-CW p=0.005.

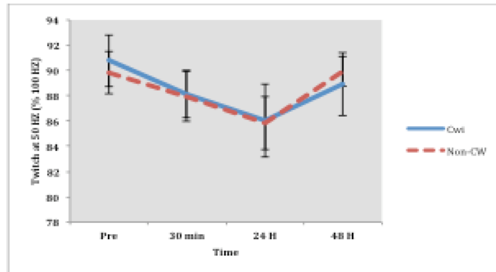


Figure 6.13 Changes in isometric twitch torque at 50 hz. Changes in isometric twitch torque after muscle damaging exercise: (CWI p =0.112, Non-CW p=0.112).

6.6.7 Effect of Recovery Type on Muscle Strength

Isokinetic maximal strength was assessed at different time points using a series of 7 different velocities of concentric contractions.

In the CWI group, results of repeated measure ANOVA showed a significant effect on recovery time at all velocities tested from 60 – 300°/s. At 300°/s (p=<0.001), torque was decreased 15 minutes after cold-water immersion (P=0.007) and remained at the same low-level 24 hr later (P=0.007), but had recovered by 48 hr (p=0.046). The other speeds also showed significant reduction in torque at 60°/s (p= 0.046), 120°/s (p=0.045), 180°/s (p= 0.015), 240°/s (p=0.031). There were no significant differences at 360°/s (p= 0.054), 420°/s (p=0.395).

In the non-cold water group, results of repeated measure ANOVA demonstrated a significant (P=<0.001) effect on torque at velocities 60°/s (p= 0.045), 120°/s (p=0.045), 240°/s (P=0.001) and 360°/s (p= 0.032). At 240°/s, torque was decreased 15 minutes after cold-water immersion (P=0.001) and remained lower 24hr later (P=0.008) and 46 hr later (P=0.049). Torque at 180°/s (P= 0.289) 300°/s (P= 0.575) and 420°/s (P=0.099) were not significantly different at any time point.

There was no significant difference between groups in force production at any point. However, there were significant group * time interactions for 120°/s (p=0.001), 240°/s (p=0.001) and 360°/s (p=0.004).

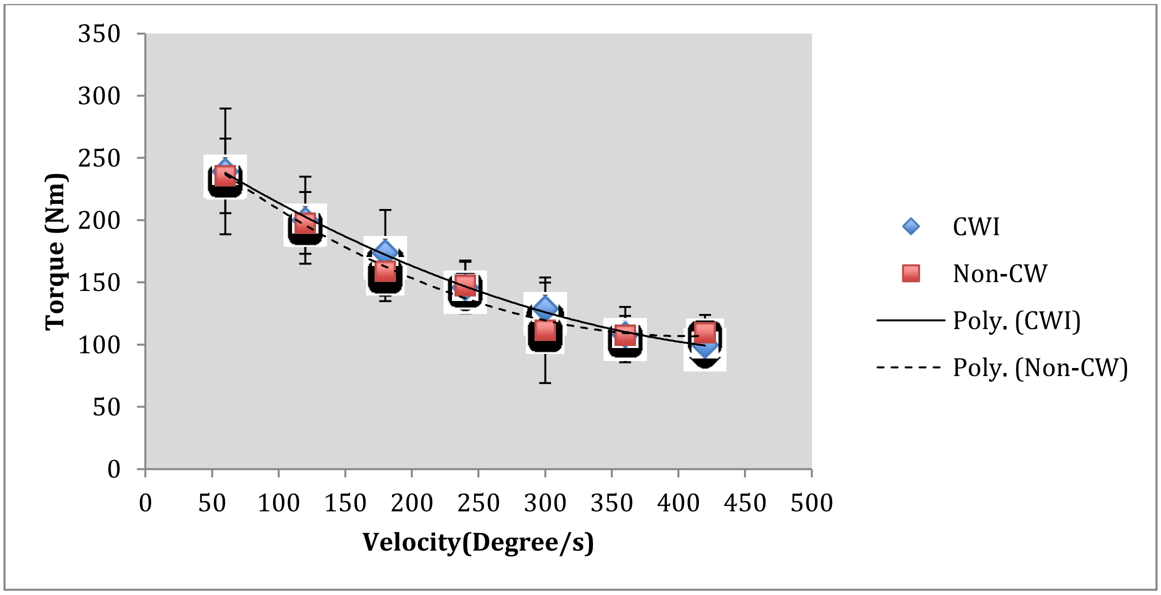


Figure 6.14 **Isokinetic Knee Extension torque at baseline between CWI and non-CW group**

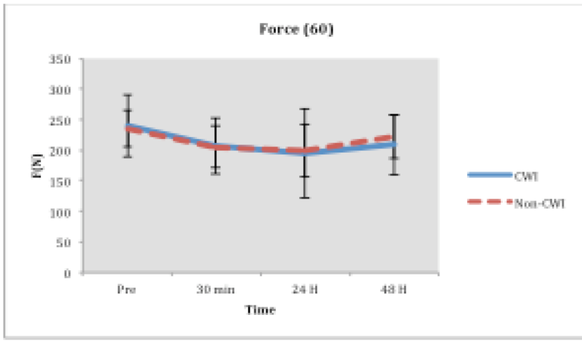


Figure 6.15 Force at 60 degree/ second (N) between groups

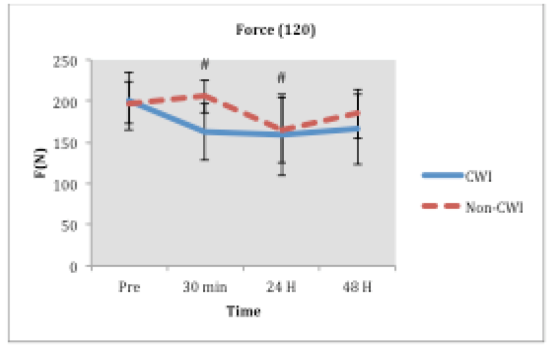


Figure 6.16 Force at 120 degree/ second (N) between groups. (#) Indicates significant difference at 30 min (p=0.026), 24h (p=0.002)

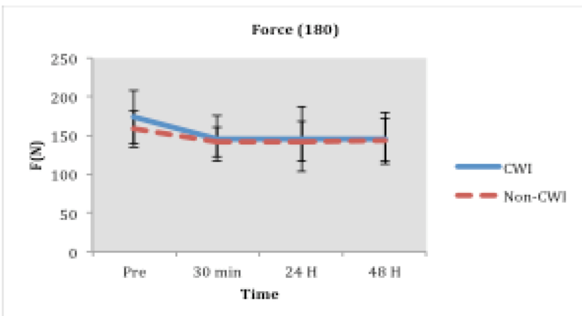


Figure 6.17 Force at 180 degree/ second (N) between groups.

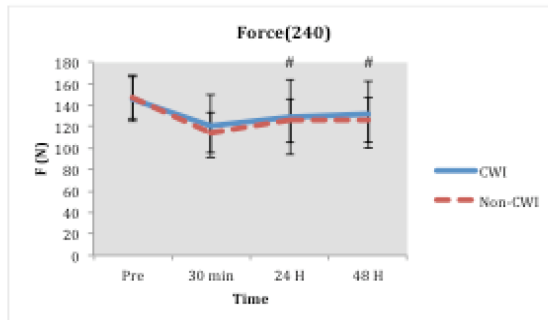
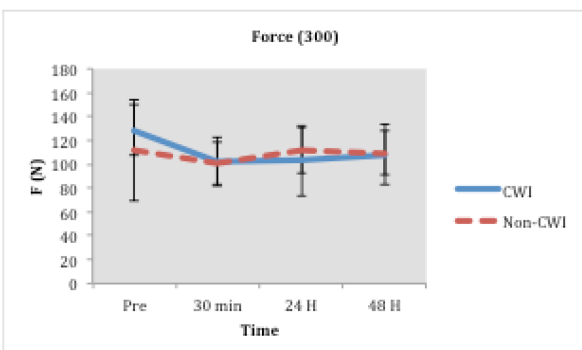


Figure 6.18 Force at 240 degree/ second (N) between groups.(#) Indicates significant difference at 24h (p=0.005) and 24h (p=0.012)



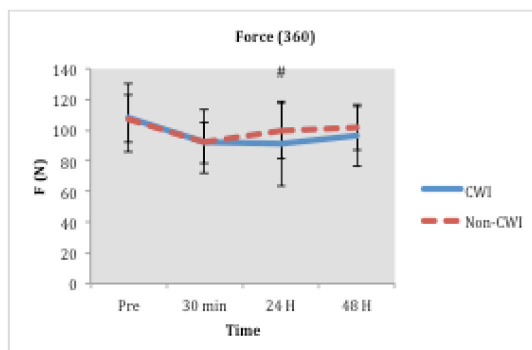


Figure 6.20 Force at 360 degree/ second (N) between groups. (#) indicates significant difference at 24h (p=0.017)

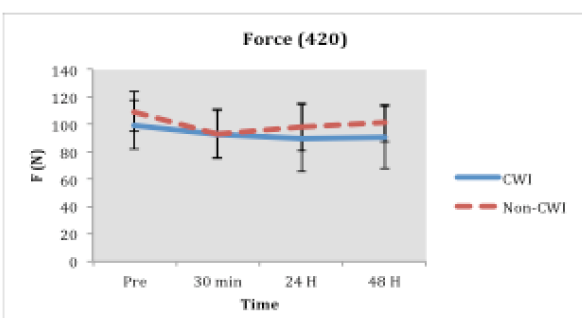


Figure 6.21 Force at 420 degree/ second (N) between CWI (p=0.395) and non-CWI group (0.099)

6.8 Discussion

The present study aimed to examine the effectiveness of immediate post exercise cold-water immersion on muscle strength, cardiovascular measures (HR, BP) and pain measured using PPT and the VAS, which are associated with DOMS following the lower extremity eccentric exercise step test in professional athletes. The CWI condition had a reduction in pain intensity at the proximal site of the quadriceps 30-minute post exercise recovery compared to the non CW condition. Conversely, the quadriceps muscle pain intensity increased in the non CW group, which was shown in the measurements up to 48hr post-CWI. However, there was no noticeable variation in perceived soreness between groups, which suggests that CWI is not an effective strategy for recovery from soreness using the VAS following eccentric exercise. Additionally, similar findings have been found in female athletes that used a similar design to the present research (Jakeman et al., 2009).

The finding that muscle soreness peaked the day following eccentric exercise is consistent with the literature (Clarkson and Hubal, 2002). The goal of decreasing pain and restoring strength should be a primary aim for a recovery session, as they both have a direct impact upon performance. A number of studies proposed that CWI has a positive effect on an individual's perception of soreness (Bailey et al., 2007; Ingram et al., 2009; Ascensao et al., 2011), possibly due to diminished sensory nerve impulses associated with tissue cooling (Analgesia effect) (Wilcock et al., 2006). In the present study, both groups demonstrated that 20 mins of eccentric step exercises with a weighted jacket induced

DOMS, which persisted during the 48hr recovery period, consistent with previous research showing that acute effects of exercise can persist throughout the ensuing recovery period (Jakeman et al., 2009). Although post-eccentric exercise CWI had no effect on alleviating muscle soreness at both 24 and 48 hour when measured using VAS, the pain pressure threshold was lower in the non CWI group 30 mins after eccentric exercise. The results of (Leeder et al., 2011), suggested that CWI did not reduce DOMS at 24hr post eccentric exercise, although it had a moderate effect at 48hr post eccentric exercise. There are very mixed reports about the effectiveness of CWI to alleviate muscle soreness, with some showing no effect (Eston and Peters,1999; Glasgow et al., 2014; Moreira et al., 2015) and others showing positive effects (Ingram et al., 2009; Elias et al., 2012; Takeda et al., 2014)Bailey et al. (2007). These contrasting results may be due to different durations, temperature, immersion depths, and frequency of application. For instance, the studies by Elias (2012) and Takeda (2014) incorporated team sport exercise, whereas the current study utilised individual eccentric exercise. Additionally, Takeda et al (2014) immersed the entire body, only excluding the neck and head at 15 degrees for 10 min; Elias immersed the participants to the xiphoid level; while Ingram used 10 degrees water temperature and the duration of 2min*5min.

Using the algometer to test sensitivity to external force applied to the vastus lateralis, we found that the proximal site of the non-cold-water group experienced significantly more pain 30 min post eccentric exercise than the cold-water group. This conflicts with the reports from another study that used the same exercise methods and measurement tools, as their CWI group experienced more pain post 24 h than the control group (Sellwood et al., 2007). However, subjectivity of pain sensations may account for the variability in the soreness measurements between studies (Corbett et al., 2012). Furthermore, both physiological and psychological influences determine the actual experience of pain, and the meaning or context that participant attach to a particular stimulus, which may influence the interpretation of the pain they experience (Sellwood et al., 2007). It was stated that a noxious cold stimulus such as CWI is known to evoke varied sensory experiences in humans including cold, pain, ache and prickling, which are mediated by thermoreceptors and nociceptors. Additionally, temperature change is associated with further perceptions of pain and aches, as well as cold, as peak pain sensation occurs at a temperature of approximately 3°C for at least 10 seconds.

Prolonged loss of strength after eccentric exercise is considered to be one of the most reliable indirect measures of muscle damage in humans (Clarkson and Hubal, 2002).
In the present study there was no apparent effect of CWI on the recovery of knee extensor MVC. Similarly, other studies have found the same results that did not present a change of strength post CWI following EIMD (Howatson et al., 2005; Sellwood et al., 2007; Jakeman et al., 2009; Corbett et al., 2012). The effect from the lack of treatment in the current study may be specifically due to the eccentric exercise protocols applied to the leg muscles that induce low levels of muscle damage, which are reflected by low pain scores, small percentage strength deficits. Previous studies have reported that CWI may facilitate the recovery of isometric muscle strength 48hr post-exercise training (Bailey et al., 2007; Vaile et al., 2008; Ingram et al., 2009). (Peiffer et al., 2009) noted a 13% decrease in knee extension MVC compared to the control group following 20 minutes of CWI at 14ho in a post-graded exercise test. Accordingly, it has been suggested by Peiffer that the further strength loss observed in the study following maximal intensity exercise is due to the

decrease in the contractile ability post-EIMD (Nybo and Nielsen, 2001). Nevertheless, the researchers only measured MVC at 90-minutes post intervention. Bailey et al. (2007) examined a longer duration of post-exercise effects and reported beneficial effects of using CWI on the recovery of muscle strength and power after intermittent shuttle-running up to 164 hours post-CWI. However, effects were evident in the knee flexors, not the knee extensors (Bailey et al. (2007)). Ingram et al. (2009) have noted that leg strength returns to the baseline within 48hr post-exercise for leg extension and flexion after cold-water immersion. However, it was reported that strength loss was observed at 48hr following contrast and passive recovery. Moreover, (Pournot et al., 2011 and Minett et al., 2013) revealed that recovery speed increased following CWI post-exhaustive intermittent exercise through MVC (Pointon et al., 2011). Likewise, a recent study by (Pournot et al., 2011) revealed significant improvements in MVC after 30 seconds of CWI and contrast water therapy (CWT) groups. This happened 1-hour after a 20-minute exhaustive intermittent exercise, and the MVC level is in contrast to the pre-exercise results. A study by (Burke et al., 2000) stated that cryotherapy causes a greater improvement in force production when applied prior to isometric training, although the result was based on laboratory settings not post-EIMD. Therefore, it is difficult to conclude that CWI impacts upon force production. The differences in results between studies may stem from variations in exercise models or variations in employed water immersion protocols. Overall, CWI seems to be most effective following higher intensity exercise and a colder temperature (10°C) than that utilised in the current study.

There is a possibility that variation between reported effectiveness of CWI on strength recovery could be due to motivation or willingness of the research participants to perform maximal voluntary contractions. In the present study, this was circumvented by applying electrically-induced contractions. No previous study has applied 7 different frequencies of electrically stimulated contractions, or 7 different force speeds, to assess contractile properties of muscle function. The results showed that force production was impairmed after 24 hours in both conditions, indicating no effect of CWI. Several studies have reported that dynamic contractile force immediately decreases on the application of a variety of cryotherapeutic modalities (Crowe et al., 2007; Bailey et al., 2007; Patterson et al., 2008). However, the reduction in the muscle's force was also observed in the control group after passive recovery. Therefore, CWI may potentially not be superior to normal passive recovery following eccentric exercise, as it did not provide a significant improvement in isokinetic strength in comparison to the control group. Differences between studies are likely related to different exercise protocols and duration (eccentric exercise in the leg, shuttle running, team games), which may have an impact on the result as well as the majority of participants in the DOMS studies were untrained (Montgomery et al., 2008; Ingram et al., 2009; King and Duffield, 2009; Rowsell et al., 2009).

Leeder et al. (2011) stated that CWI appears to have more effective analgesic properties following high intensity exercise than eccentric based exercise, which may explain the difference in the findings relating to soreness and strength. Moreover, it is possible that participants anticipated a positive outcome from CWI, which thereby may have influenced the measure of DOMS and caused a placebo effect. In the study by Broatch et al (2014) participants in thermonutral water condition were effectively deceived and led to believe that a placebo was as effective as CWI in promoting recovery from high-intensity exercise.

The increase in the HR during the first minute post-immersion persisted for five minutes, followed by a gradual decrement until it reached its baseline after ten minutes. These results were consistent with other studies which showed an increase in HR from post-immersion states (Mantoni et al., 2007; Rowsell et al., 2011). In contrast, though, other studies found that CWI decreases HR from the pre-immersion level (Crow et al., 2007; Parouty et al., 2010). A similar finding has been reported by Crow et al. (2007) that a reduced muscle blood flow and temperature causes a reduction in the heart rate. The present study showed no change in systolic or diastolic blood pressure post CWI during the period of measurements. This could indicate that either hydrostatic pressure or temperature had not reached the levels needed to affect the cardiovascular function (for more detailed discussion, see chapter five).

6.9 Limitations

Neither the blood pressure nor heart rate was measured throughout the study in the control group, due to the lack of apparatus. Hence, the impact of passive recovery on these variables remains unknown. Secondly, the heart rate and BP were not measured pre-exercise, which could have provided actual unaffected resting measurements. Furthermore, muscle temperature was not assessed during any of the recovery protocols in this study.

6.10 Conclusion

These results indicate that a single bout of cold-water immersion at 12°-13°C for 15 minutes may not be superior to normal passive recovery in preventing or reducing the intensity of DOMS symptoms, but there were not negative effects presented either. Similar to other research in this area, the effect of CW therapy is still controversial and further studies are needed to elucidate the effect of cold-water.

Chapter Seven: Effectiveness of Cold-water Immersion on muscle damage and inflammatory marker post delayed onset Muscle Soreness

7.1 Introduction

Muscle damage is one specific direct result caused by high intensity eccentric exercise that commonly presents itself in the field of sport (Nosaka and Newton, 2002). Meneghel et al. (2013) defined muscle damage as a detrimental characteristic caused by disruptions of ultrastructural sarcomeric proteins, degeneration of muscle proteins, inflammation and intramuscular proteins being released into the bloodstream. Moreover, muscle damage and physical injury that are initiated through exercise are believed to create an acute-phase local inflammatory response, which generally incorporates the release of certain cytokines that control individual immune responses (Chen and Hsieh, 2000). Consequently, muscle repair and regeneration are subsequently enhanced through the initiation and moderation of the inflammatory response (Malm, 2001, Montgomery et al., 2008a). Additionally, Interleukin- 1β, and -6 (IL- 1β, IL-6) have been comprehensively researched within the acute inflammatory response and it was ascertained that the levels became heightened in the hours post-eccentric exercise (Steensberg et al., 2001).

It has been denoted that post-eccentric exercise, several cytokines may be distinguished within plasma (Pedersen, 2000). Invariably, following this manner of exercise, the concentrations in plasma of interleukin-1 receptor antagonist (IL-1ra), monocyte chemotactic protein (MCP)-1, and the granulocyte-colony stimulating factor (G-CSF) become more excessively enhanced. Comparatively, the alterations from concentrations in plasma of IL-8, IL-10 and IL-12 are distinctly more variable. Furthermore, the plasma concentrations of IL-1β, IL-2, IL-5, IL-13, IL-15, IL-17, TNF-α and interferon (IFN)-γ have been shown to remain constant without alteration following eccentric exercise (Paulsen et al., 2012). Thus, the entire group of defined cytokines are characterised as either anti-inflammatory to promote inflammation, or pro-inflammatory to inhibit inflammation (Pedersen, 2000). The role of cytokines in muscle damage is shown in Figure 7.1

Cytokines	Pro-inflammatory	Anti-inflammatory	Degenerative	Regenerative	Fibrogenic
IL-17F	✓				
GMCSF	✓				
IFNY	✓				
IL-10		✓		✓	
CCL20MIP3a					
IL12p70					
IL-15	✓				
IL-17	✓				
IL-22	✓				
IL-9	✓				
IL-1β	✓				
IL-33	✓				
IL-2	✓				
IL-21		✓			
IL-4		✓		✓	
IL-23	✓				
IL-5		✓			
IL-6	✓	✓	✓	✓	
IL17EIL25					
IL-31	✓				
TNF α	✓		✓	✓	✓
TNFb	✓				✓

Figure 7.1 Roles of cytokines and inflammatory in muscle damage (Philippou and Koutsilieris, 2012)

It has been conveyed by Smith et al. (2008) that certain pro-inflammatory cytokines release TNF-α, IL-1β and IL-6, which are part of regeneration and responsible for the stimulation and activation of macrophages that follow muscle-damaging eccentric exercise, as well as during the acute inflammatory phase. Therefore, the tissue repair and regeneration is orchestrated by controlled release of cytokines, while the removal of debris is promoted by macrophages through the release of various growth factors, chemokines and cytokines (Butterfield et al., 2006). In addition, the initial inflammatory response is attenuated by the release of growth factors and anti-inflammatory cytokines, such as IL-4 and IL-10 through different macrophages. Hence, the conclusive route within the process of inflammation is relative to a maintained balance between pro-inflammatory cytokines (i.e TNF-a and IL-1b), and anti-inflammatory cytokines (i.e IL-4 and IL-10); this ultimately proceeds to the damaged tissue becoming sufficiently repaired, while not inducing an excessive inflammatory response (White and Wells, 2013). Similarly, through circulation during the process of exercise, IL-6 is generally selected as a general 'marker' of cytokine responses, due to its role in inflammation and repair of muscle tissue (Toumi et al., 2006).

The capacity to measure the inflammatory responses during exercise and recovery has been used to evidence the implementation of CWI, although the evidence supporting its effectiveness at reducing inflammation is lacking (Halson et al., 2014; Leeder et al., 2015) and previous investigations have been inconclusive (Takeda et al., 2014; Baily et al., 2007; Banfi et al., 2007).

In general, both exercise and cold temperatures are physiological stressors, requiring homeostatic adjustments, some of which include alteration of the concentrations of circulating hormones or cytokines (Steensberg et al., 2003). For example, the steroid hormone Cortisol is released by the body in response to stress factors that can be either physical or psychological, which may function as an anti-inflammatory agent through the suppression of the immune system (Kandhalu, 2013). Specifically, maximum intensity exercise or long duration activities are considered factors of stress, which can increase the concentration of cortisol (Coelho et al., 2013). Indeed, Halson et al. (2008) have noted that a decrease of Cortisol is associated with enhanced muscle recovery, so any intervention that reduces cortisol may be beneficial for recovery from intense exercise (Lac and Berthon, 2000).

Therefore, the purpose of the current study was to understand the how the inflammatory markers are affected by CWI, as well as in regards to the stress hormone post-eccentric exercise.

7.2 Methods

7.2.1 Subjects

Eight active participants of *phase one* and eight Welsh Rugby players volunteered to participate in this study. Their demographic is present in the method chapter (Chapter 4).

7.2.2. Study design

The research was particularly structured to measure the influence of CWI on acute inflammation and stress hormone following step-up activity. Throughout the study, during their period of fasting the subjects provided blood samples before muscle-damaging exercise that involved performing a step-up exercise for 20 minute (as previously detailed in chapter 4). Upon completion of the exercise, participants were randomly assigned to one of recovery interventions for 15 minutes: CWI or passive recovery. Following the recovery period, participants immediately provide a blood sample, as well as 24 and 48 hours post-intervention.

7.2.3 Blood sampling

A venous blood sample of ~8 ml was obtained from a vein in the antecubital fossa using a 21 gauge needle and a vacutainer system. Samples were individually coded for each participant and were centrifuged within 30 minutes of collection at 2000 x g to separate the plasma from the remaining blood constituents. Blood samples were allocated and then stored at -80 °C until the process of analysis.

7.2.4 Biochemical Analysis

Using commercially available Assay Kits (Milliplex map Kit), the levels of cortisol (7-Plex Human Neuropeptide Magnetic Kit) and the inflammatory cytokines (Human Th 17 Magnetic Bead Panel) was outlined. To eliminate any discrepancies in the analysis conditions, each examination was conducted twice. Every analysis adhered purposely to a certain number of stages, as highlighted by the product's standard manufacturer procedures. In order to examine the serum correctly, the frozen blood was thawed and left to reach the room temperature, before subsequently being mixed well by vortexing and centrifuge prior to use in the Assay. Following this procedure, 7 standards were combined, diluted and arranged into 96-Well Plates. Also, the samples were arranged in a replicable manner. Likewise, the Assay procedure for the reagents preparation and incubations time for both enzyme analysis provided with kit were followed. Once the procedure was completed, all the plates were then placed in a plate reader, where the neuropeptide and cytokine levels were calculated.

7.2.5. Data Analysis and statistical consideration

The Statistical Package of the Social Science (SPSS) for windows (v 21, Armonk, New York 10504-1722, USA) was used for analysis. Information was distinguished as average and standard deviation, while statistical significance was set as $\alpha = 0.05$. A Repeated Measures Analysis of Variance (ANOVA) was utilised. The values for both the cortisol and cytokines are determined as mean and standard deviation as the data is normally distributed.

7.3 Findings of Blood Data

7.3.1 Phase One – Active Group

No variables were evident through the progressive measurement stages of hormone concentration levels.

7.3.2.Phase Two – CWI and Passive Recovery Group

7.3.2.1 Cortisol

No significant changes in the level of cortisol were observed in response to the 15 min intervention during the test period for the two groups ($p= 0.833$). There was a significant difference in the concentration levels of cortisol over time ($p=0.049$). Compared to the baseline, concentrations at 24h ($p=0.042$) were significantly lower.

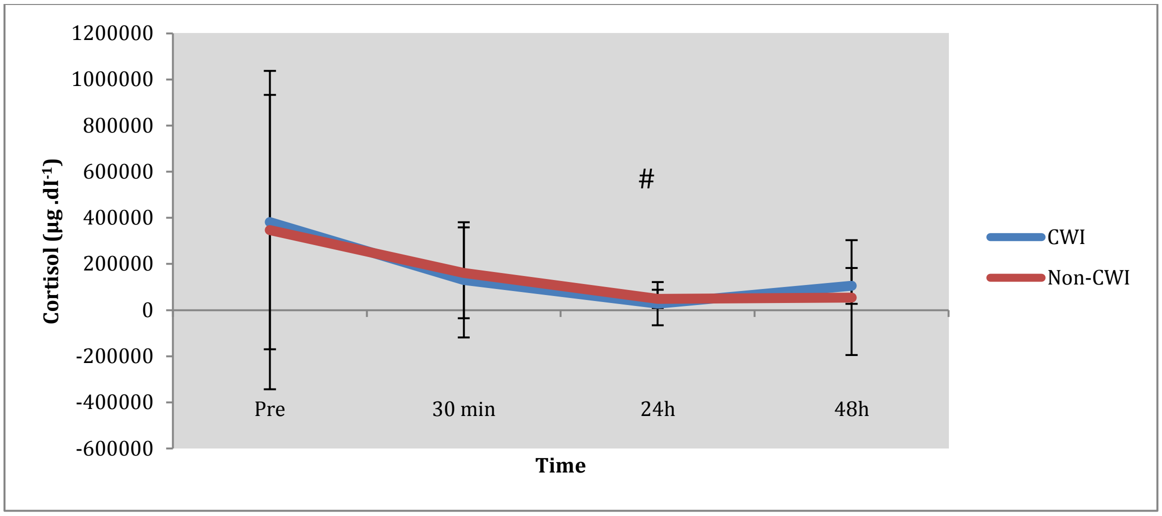


Figure 7.2 Comparison between two groups for cortisol changes through the 48h recovery period. (#) Indicates significant difference in the levels of cortisol 24h (p=0.042)

7.3.2.2. Alpha Melanocyte Stimulating Hormone (α-MSH)

Repeated Measure ANOVA indicated that there were no significant differences in the concentration of α-MSH between groups during the treatment time (p=0.918). While, there were significant differences of the levels over time (p= 0.032). The level of α-MSH 24h (p=0.025) and 48h (p=0.041) was lower than the baseline.

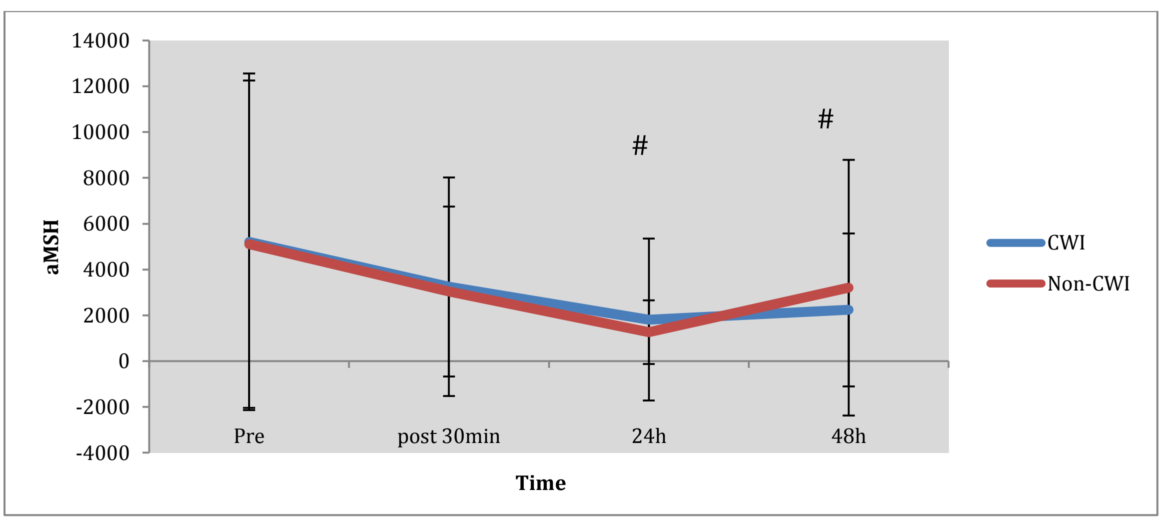


Figure 7.3 Comparison between two groups for aMSH changes through the 48h recovery period. (#) Indicates significant difference in the level of α-MSH at 24h ($p=0.025$) and 48h ($p=0.041$).

7.3.2.3 Oxytocin

Both groups did not show any changes in the level of Oxytocin neither at baseline nor over time ($p = 0.350$). Furthermore, there were no effects of time on the concentration of the Oxytocin ($p = 0.291$).

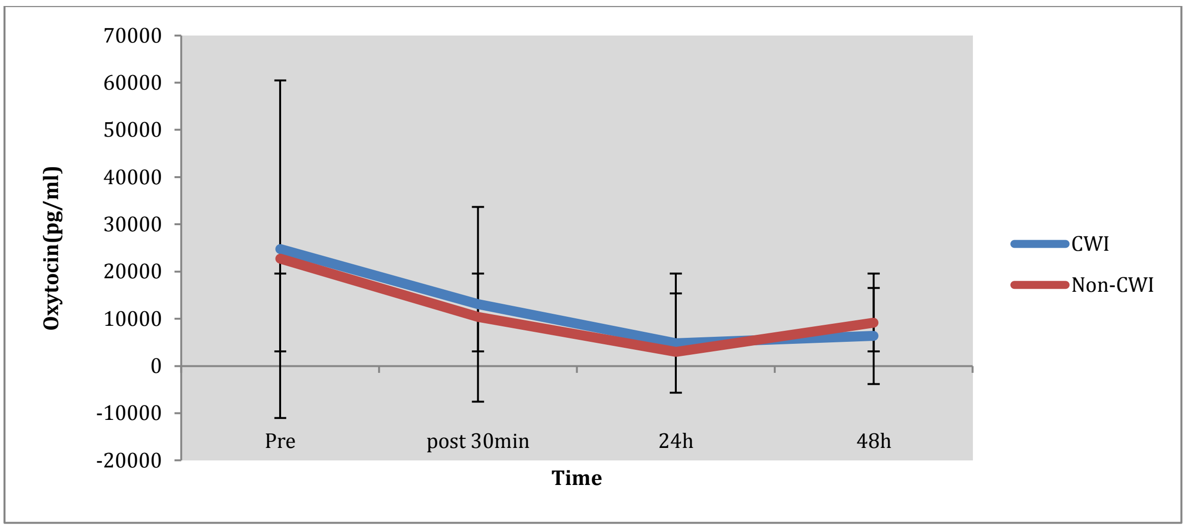


Figure 7.4 Comparison between two groups for oxytocin changes through the 48h recovery period.

7.3.2.4 β-Endorphin

Repeated measures ANOVA showed no significant differences over time ($p=0.302$) or between groups ($p=0.768$) on the level of β-Endorphin during the treatment period.

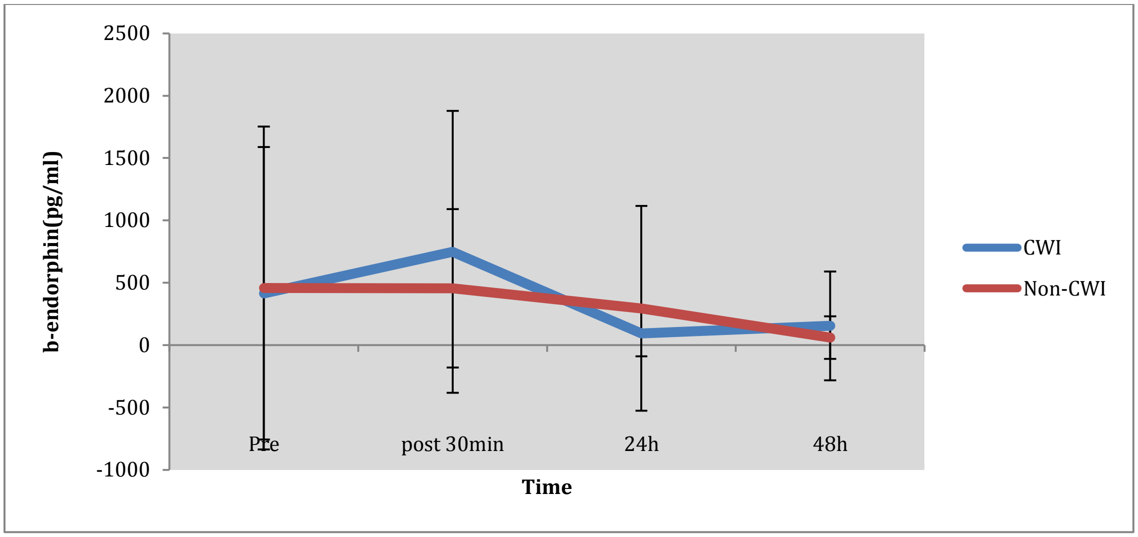


Figure 7.5 Comparison between two groups for β-endorphin changes through the 48h recovery period.

7.3.3 Anti-inflammatory Cytokines

7.3.3.1 Interleukin-6 (IL-6)

Both groups had similar levels of IL-6 at the baseline (p=0.941). The inflammatory marker IL-6 showed no significant differences between groups (P= 0.167). However, the results showed that there was a significant difference in the level of IL-6 over time (p= 0.022). Compared to the baseline, the concentration of IL-6 at 24h (p=0.013) and 48h (p=0.019) were significantly higher.

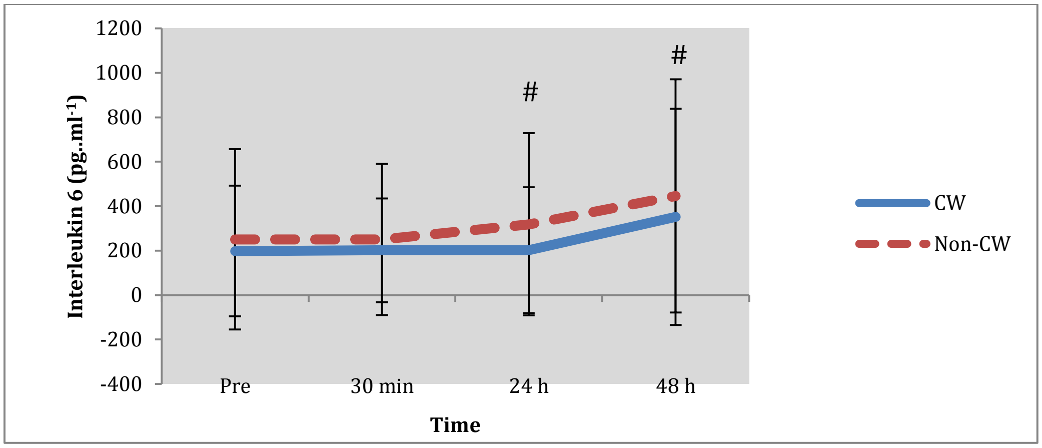


Figure 7.6 Comparison between two groups for IL-6 changes through the 48h recovery period. (#) Indicates significant differences of the level of IL-6 at 24 (p=0.013) and 48hr (p=0.019) from the baseline measurement.

7.3.3.2 Interleukin-10 (IL-10)

The repeated measure ANOVA showed no significant difference in the level of IL-10 between groups ($p= 0.839$) and no significant differences over the measurement time ($p= 0.075$).

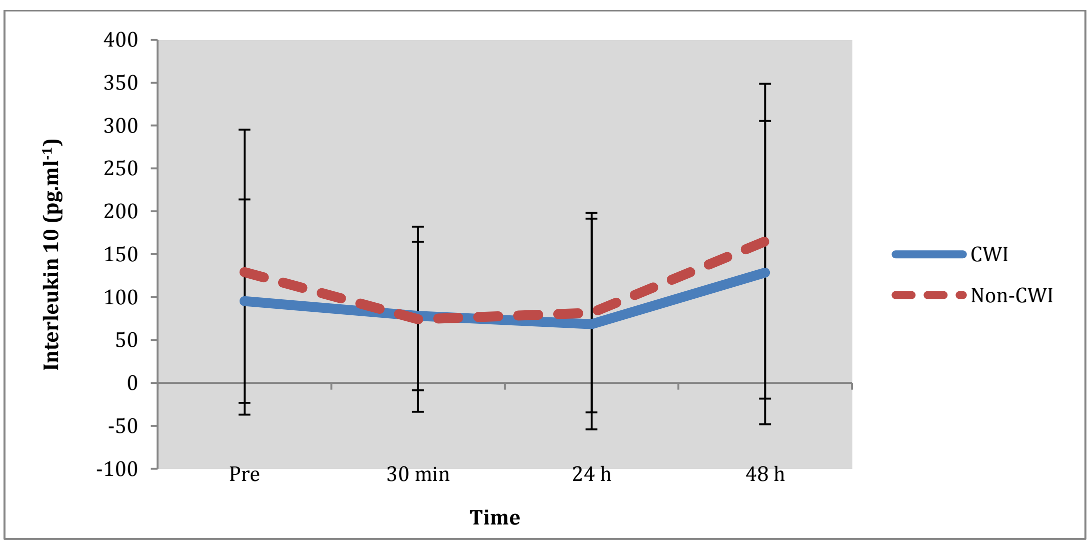


Figure 7.7 Comparison between two groups for IL-10 changes through the 48h recovery period.

7.3.3.3 Interleukin14 (IL-4), Interleukin-21 (IL-21)

The levels of IL-4 and IL- 21 showed no significant differences between groups (p= 0.389), (p=0.841) or within group over time ((p = 0.344 and p=0.217, respectively).

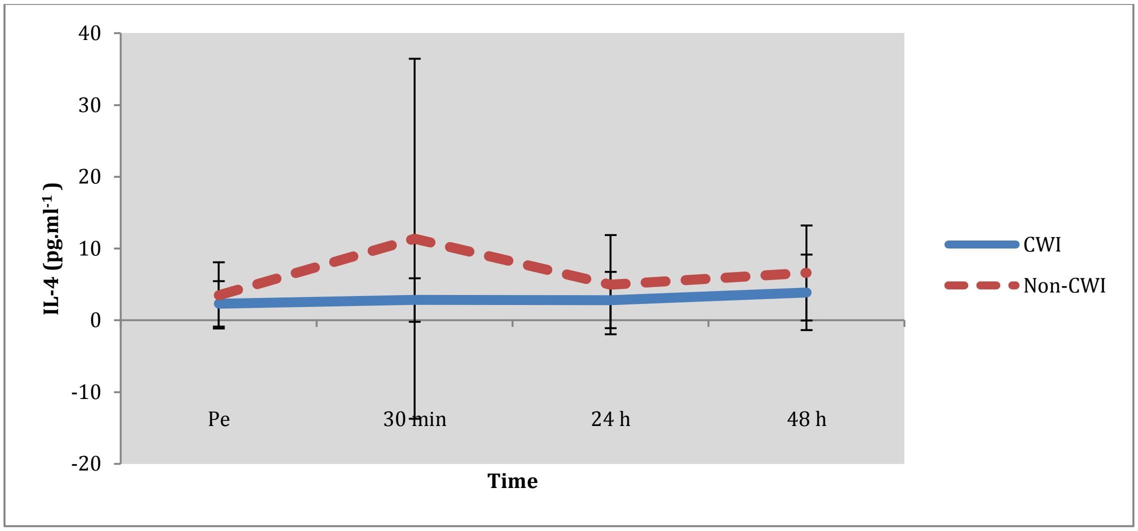


Figure 7. 8 Comparison between two groups for IL-4 changes through the 48h recovery period.

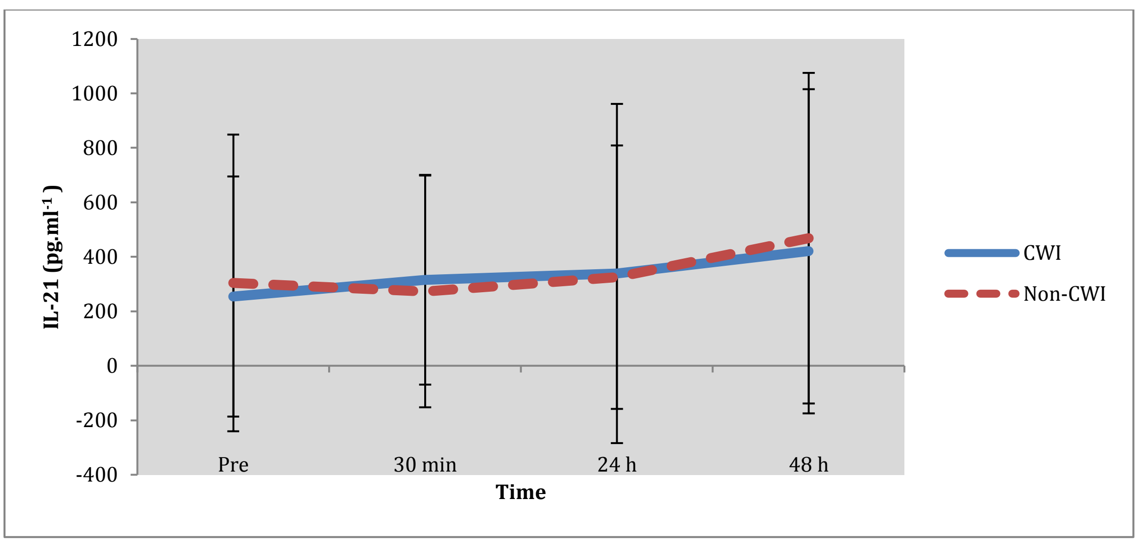


Figure 7.9 Comparison between two groups for IL-21 changes through the 48h recovery period.

7.3.3.4 Interleukin- 5 (IL-5)

The result showed no significant difference in the levels of IL-5 between groups over measurement time (p=0. 795). However, there was a change in the level of IL-5 over time (p=0.017). The level of IL-5 was increased at 24h (p=0.021) and 48h (p=0.013) compared to the baseline.

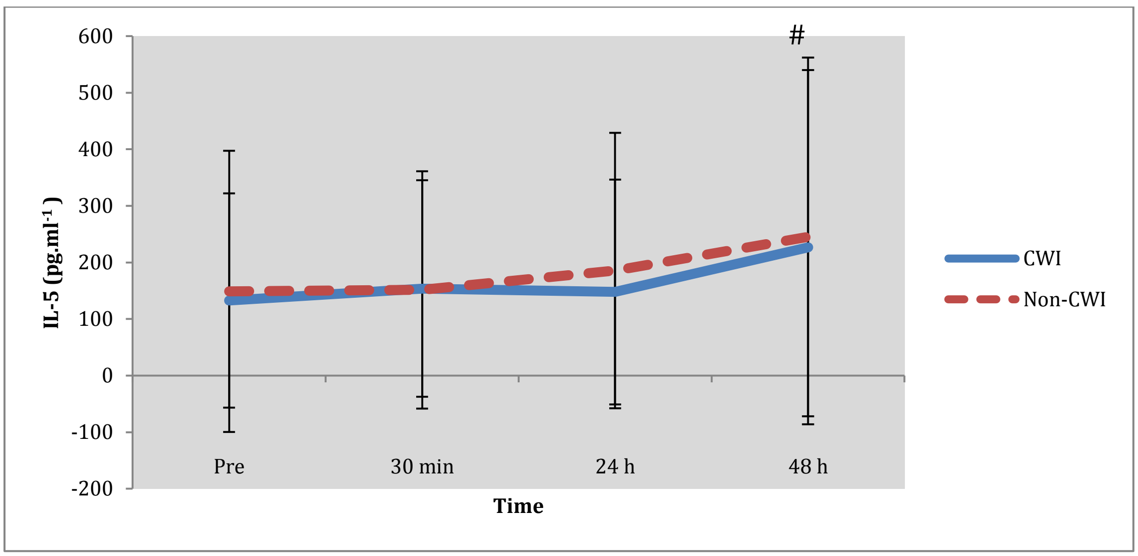


Figure 7. 10 Comparison between two groups for IL-5 changes through the 48h recovery period. (#) Indicates significant differences of the level of IL-5 at 24 (p=0.021) and 48h (p=0.013) from the baseline measurement.

7.3.4 Pro-inflammatory Cytokines

7.3.4.1 Interleukin 1β (IL-1β)

The result showed no significant differences in the level of IL1β between cold- water and non-cold water groups (p=0.940). There were no significant differences over time (p=0.130).

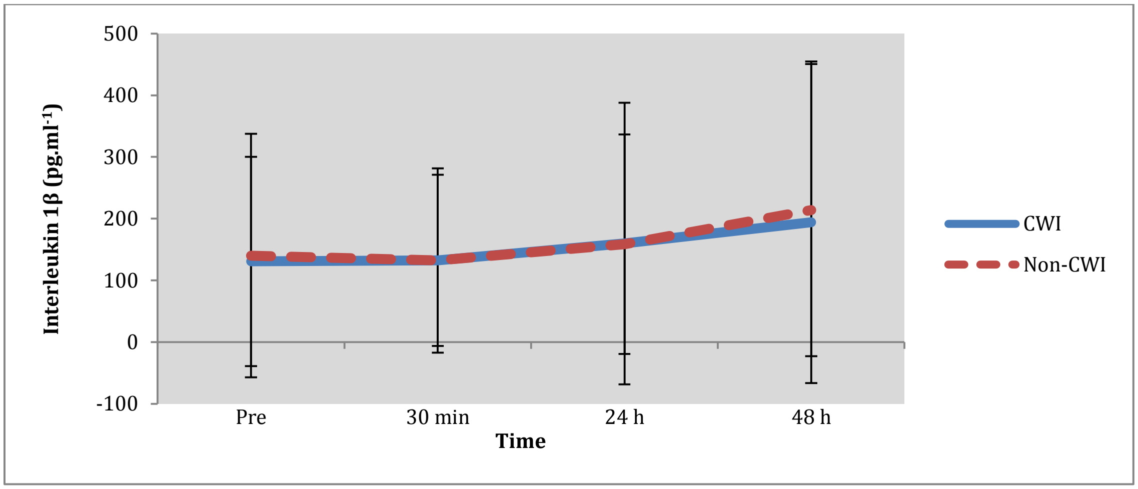


Figure 7. 11 Comparison between two groups for IL-1β changes through the 48h recovery period.

7.3.4.2 Tumor necrosis factor-α (TNFα)

The result showed no significant differences in the level of TNFα between cold water and non-cold water groups (p=0.850) over measurement time. There were no significant differences over time (p =0.061).

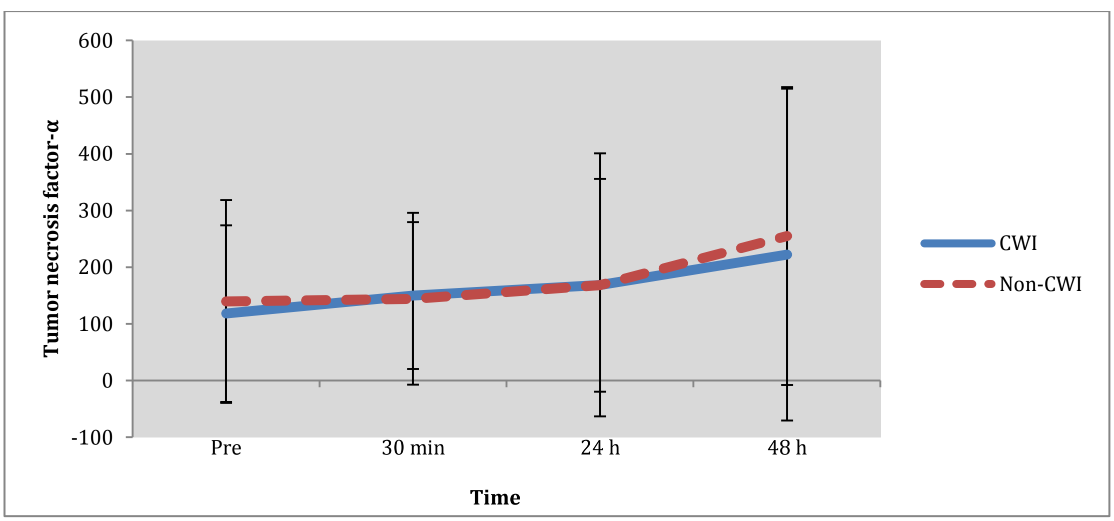


Figure 7. 12 Comparison between two groups for TNFα changes through the 48h recovery period.

7.3.4.3 Interferon Gamma (INFγ)

The result showed no significant differences in the level of INFγ between groups (p= 0.593) over measurement time. However, the repeated measure ANOVA showed a trend for changes occurring over time (p= 0.051), giving statistically significant differences in the levels of INFγ between the baseline and 48h (p=0.019).

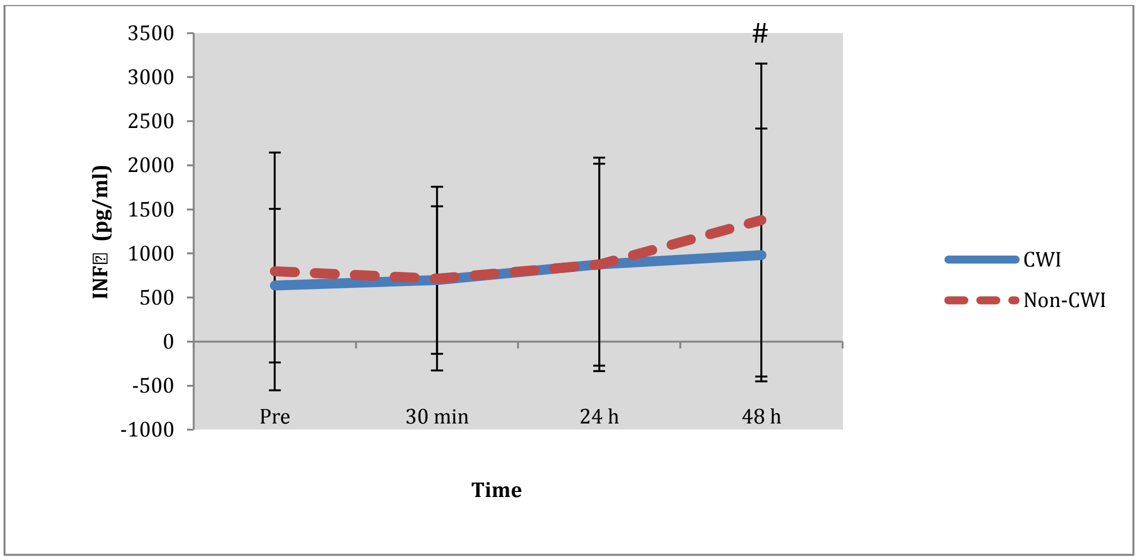


Figure 7. 13 Comparison between two groups for INFγ changes through the 48h recovery period. (#) Indicated significant differences over time in the levels of the NFγ 48h (p=0.019) from the baseline.

7.3.4.4 Granulocyte-macrophage colony-stimulating factor (GM-CSF)

There were no significant differences in the concentration level of GM-CSF between groups (p=0.556) over measurement period and no significant differences over time (p= 0.074).

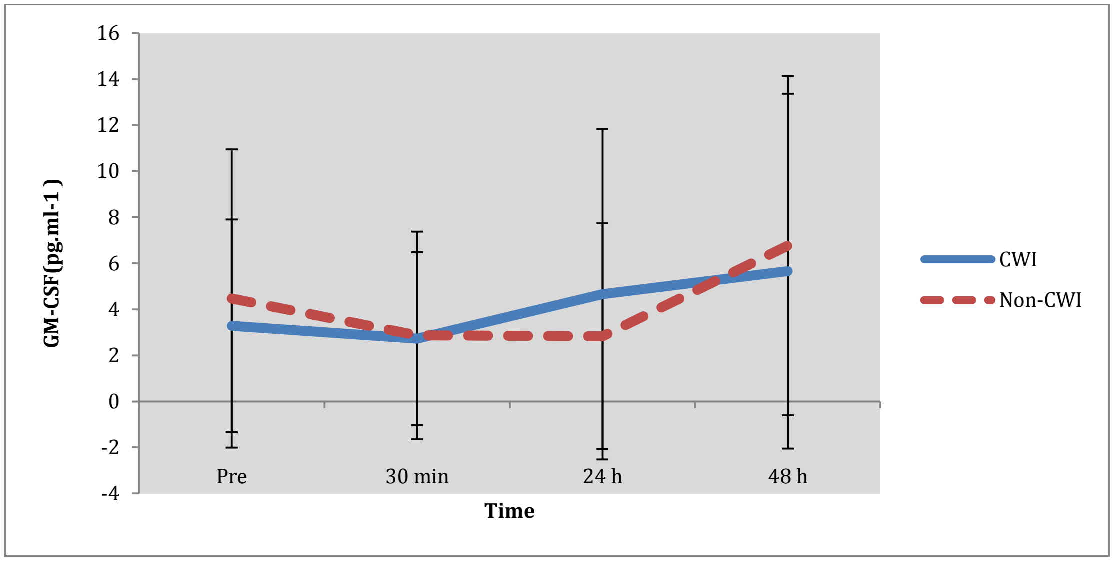


Figure 7. 14 Comparison between two groups for GM-CSF changes through the 48h recovery period.

7.3.4.5 Interleukin IL-17F

There were no significant differences in the concentration level of IL-17F between groups ($p=0.311$) over the measurement period. There were no significant differences over time ($p=0.225$).

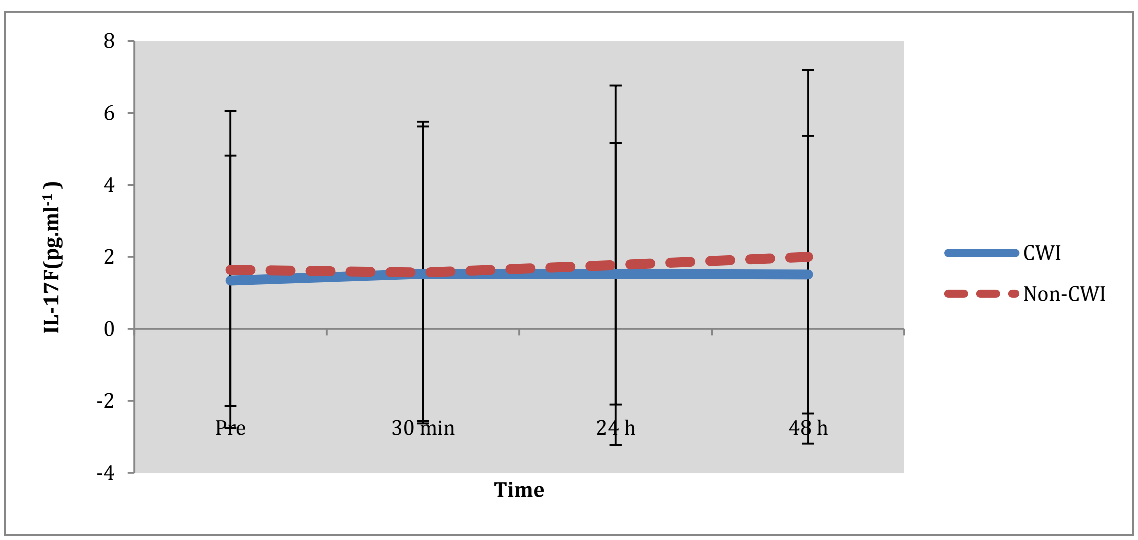


Figure 7. 15 Comparison between two groups for IL-17F changes through the 48h recovery period.

7.3.4.6 Chemokine (C-C motif) ligand 20 (CCL20)

There were no significant differences in the concentration level of CCL20 between groups (p=0.635) over measurement period. There was a significant difference over time (p=0.037). Compared to the baseline levels of the CCL20, 48h (p=0.028) showed a significant increase of the level of CCL20 over time.

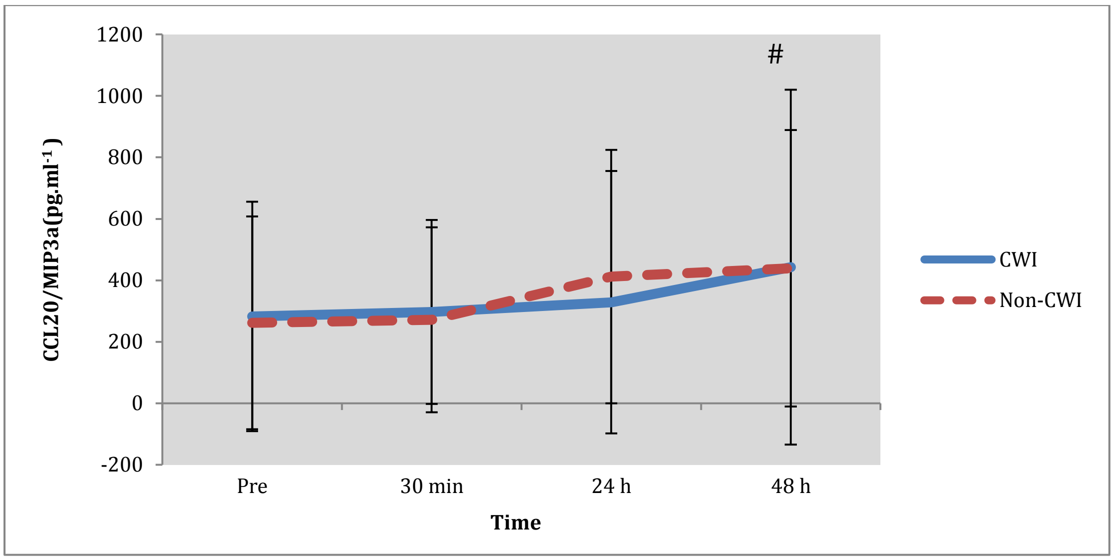


Figure 7. 16 Comparison between two groups for CCL20/MIP3a changes through the 48h recovery period. (#) Indicated significant differences over time in the levels of the CL20/MIP3a 48h (p=0.028) from the baseline.

7.3.4.7 Interleukin IL-17A

The result showed no significant differences in the level of IL-17A between groups (p=0.758) over measurement time. There was a significant difference in the level of IL-17A over time (p=0.034). The level of IL-17A at 48h (p=0.020) was statistically higher than the baseline (p=0.607) and 24h (p=0.321).

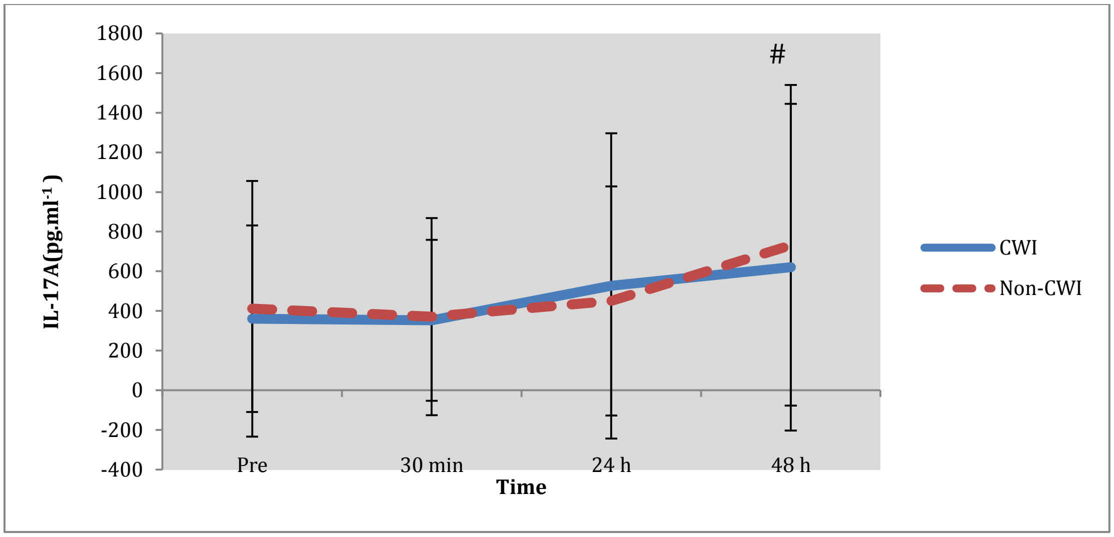


Figure 7. 17 Comparison between two groups for IL-17A changes through the 48h recovery period. (#) Indicates a significant difference in the levels of IL-17A at 48h (p=0.034).

7.3.4.8 Interleukin IL-12 (p70)

The result showed no significant differences in the level of IL-12 (p70) between groups (p= 0.878) over measurement time. There were no significant differences in the level of IL-12 (p70) over time (p=0.068).

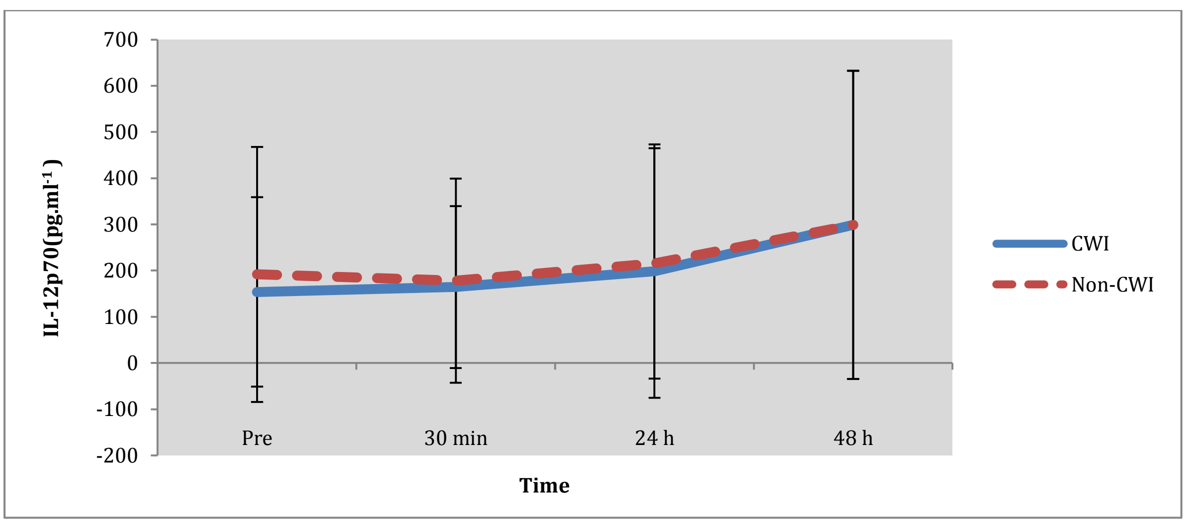


Figure 7. 18 Comparison between two groups for IL-12 (p70) changes through the 48h recovery period

7.3.4.9 Interleukin IL-22

The result showed no significant differences in the level of IL-22 between groups (p= 0.260) over measurement time. There were no significance differences over time (p=0.298).

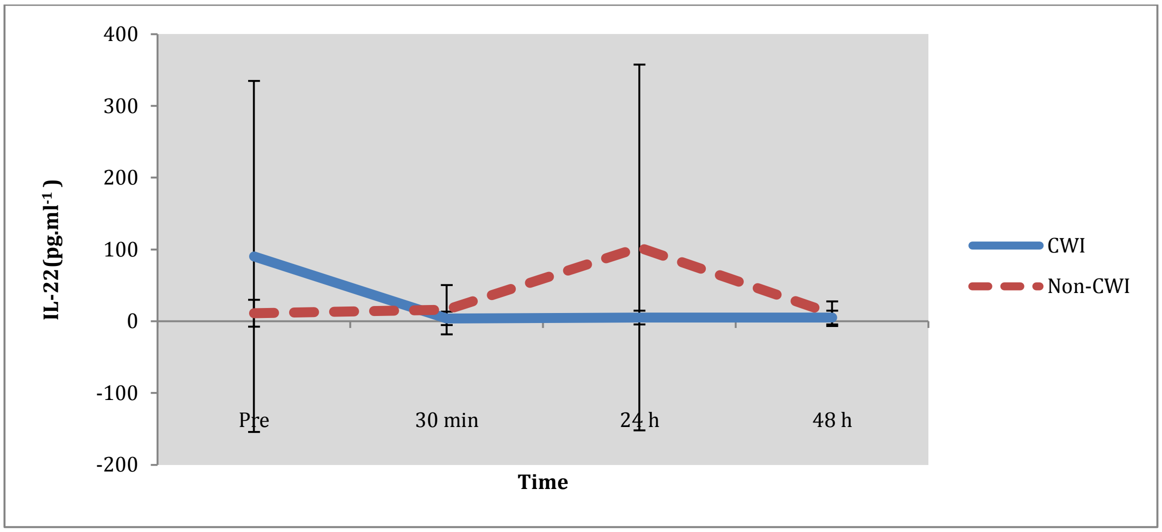


Figure 7. 19 Comparison between two groups for IL-22 changes through the 48h recovery period.

7.3.4.10 Interleukin IL-9

The result showed no significant differences in the level of IL-9 between groups (p= 0.857) over measurement time. There was a significant difference in the level of IL-9 over time (p= 0.024). The level of IL-9 was increased in 48h (p=0.016) compared to the baseline and 24h.

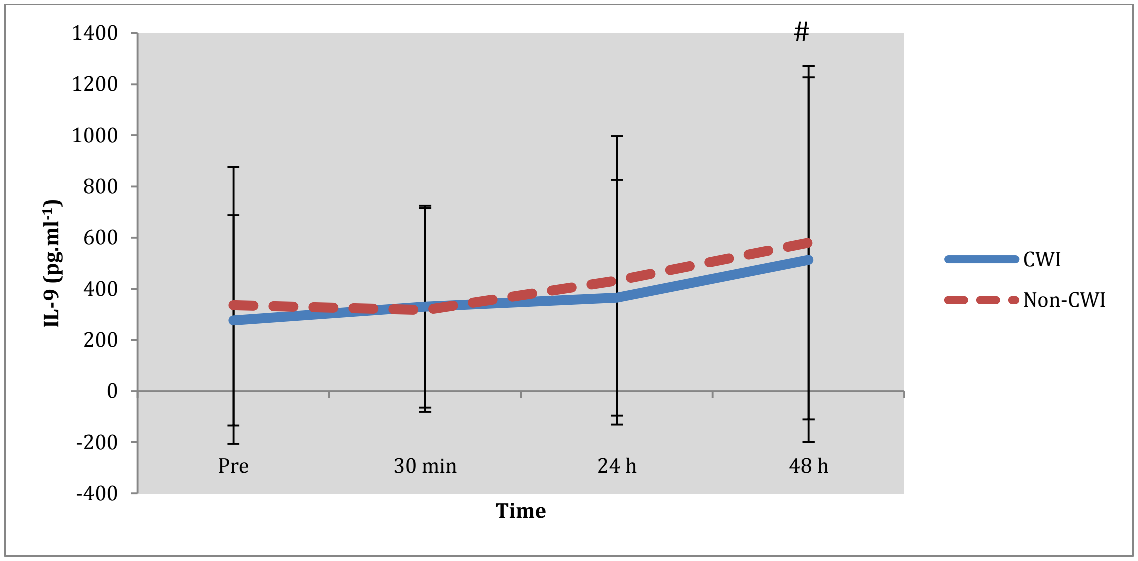


Figure 7. 20 Comparison between two groups for IL-9 changes through the 48h recovery period. (#) Indicates a significant difference in the levels of IL-9 at 48h compared with baseline (p=0.016).

7.3.4.11 Interleukin IL-33

The result showed no significant differences in the level of IL-33 between cold and non-cold group ($p= 0.778$) over measurement time. There were no significant differences over time ($p= 0.350$).

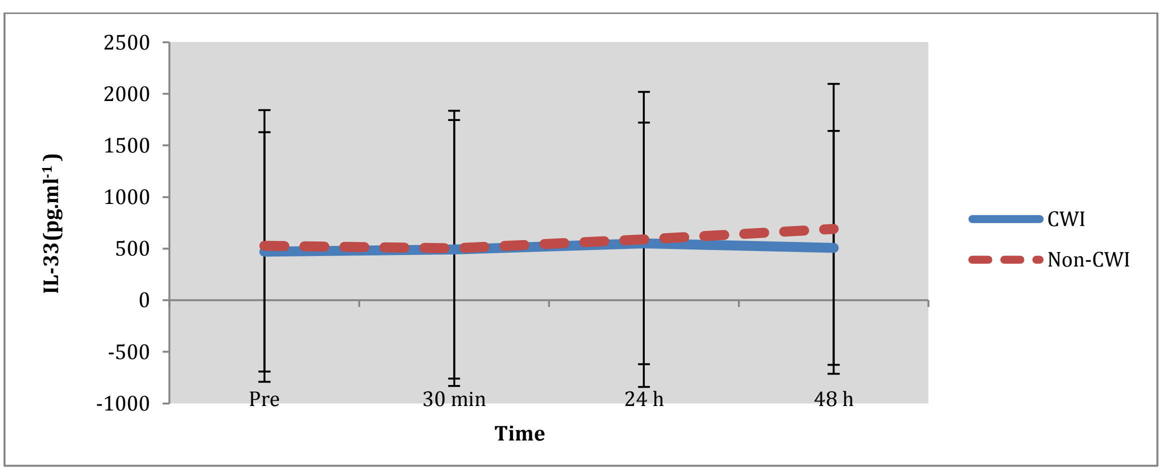


Figure 7. 21 Comparison between two groups for IL-33 changes through the 48h recovery period.

7.3.4.12 Interleukin IL-2

The result revealed that there were no significant differences in the level of IL-2 between groups (p= 0.858) over measurement time. The repeated measure ANOVA showed a significant difference in the level of IL-2 over time (p= 0.012). Compared to the baseline (p=0.577) and 24h (p=0.568), 48h (p=0.011) showed an increase in the level of IL-2.

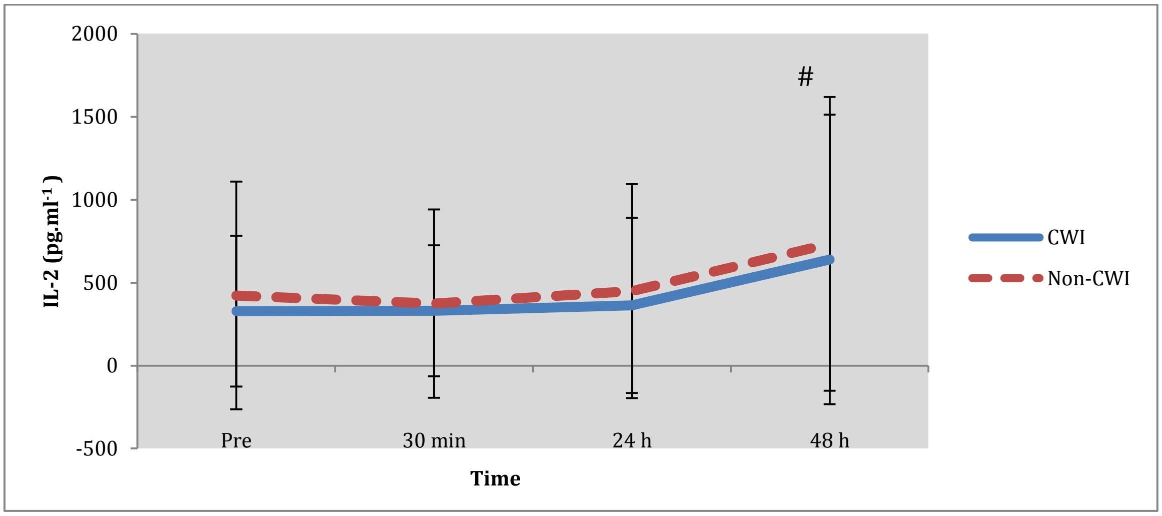


Figure 7. 22 Comparison between two groups for IL-2 changes through the 48h recovery period. (#) Indicates a significant difference in the levels of IL-2 at 48h (p=0.011).

7.3.4.13 Interleukin IL-23

The result showed that there were no significant differences in the level of IL-23 between cold- water and non-cold water group (p= 0.330) or within group over measurement time (p=0.063).

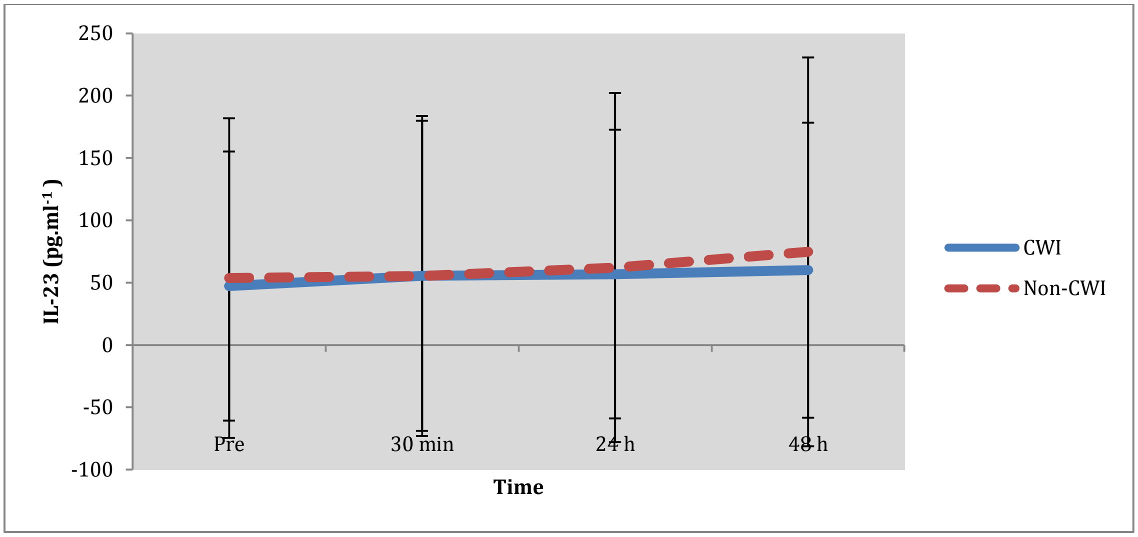


Figure 7. 23 Comparison between two groups for IL-23 changes through the 48h recovery period

7.3.4.14 Interleukin IL-17E/IL-25

The result showed that there were no significant differences in the level of IL-17E/IL-25 between groups (p= 0.396) over measurement time. There were no significant differences over time (p=0.393).

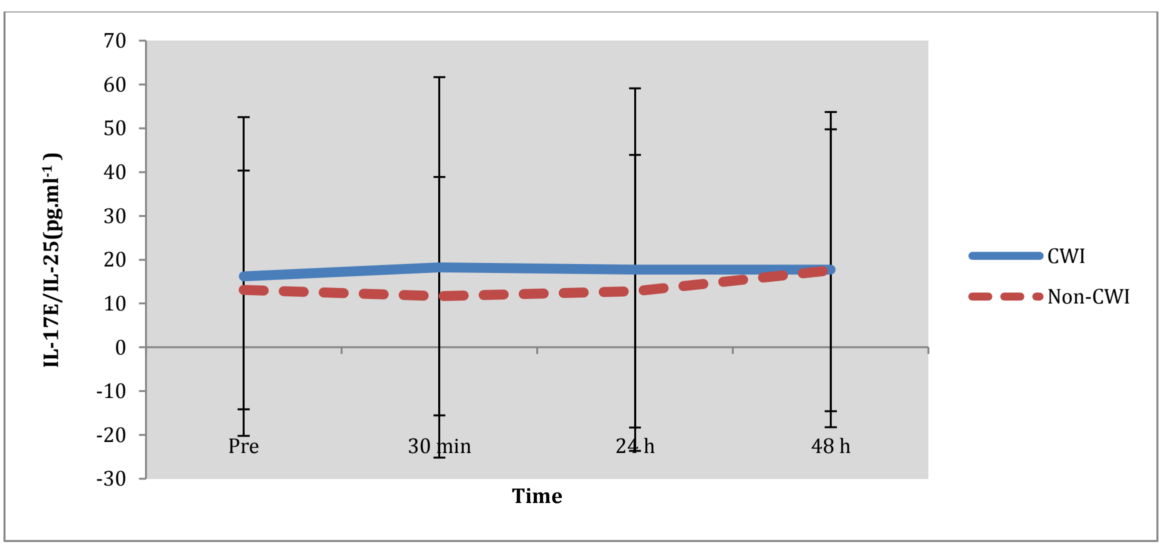


Figure 7. 23 Comparison between two groups for IL-17E/IL-25 changes through the 48h recovery period

7.3.4.15 Interleukin IL-31

Repeated measure ANOVA showed that there were significant differences in the level of IL-31 between groups (p= 0.366) over measurement time. There were no significant differences over time (p=0.419).

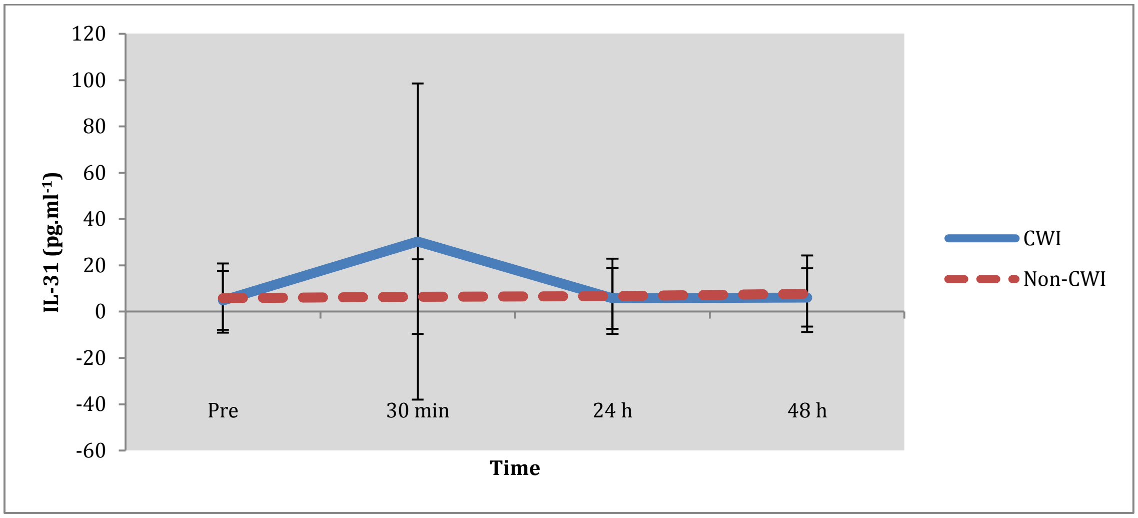


Figure 7. 24 Comparison between two groups for IL-31 changes through the 48h recovery period.

7.3.4.16 Tumor necrosis factor-b (TNFb)

The result showed no significant differences in the level of TNFb between groups (p=0.767) over measurement time. There was a significant difference over time (p=0.028). The level of TNFb at 48h (p=0.012) was different than the baseline.

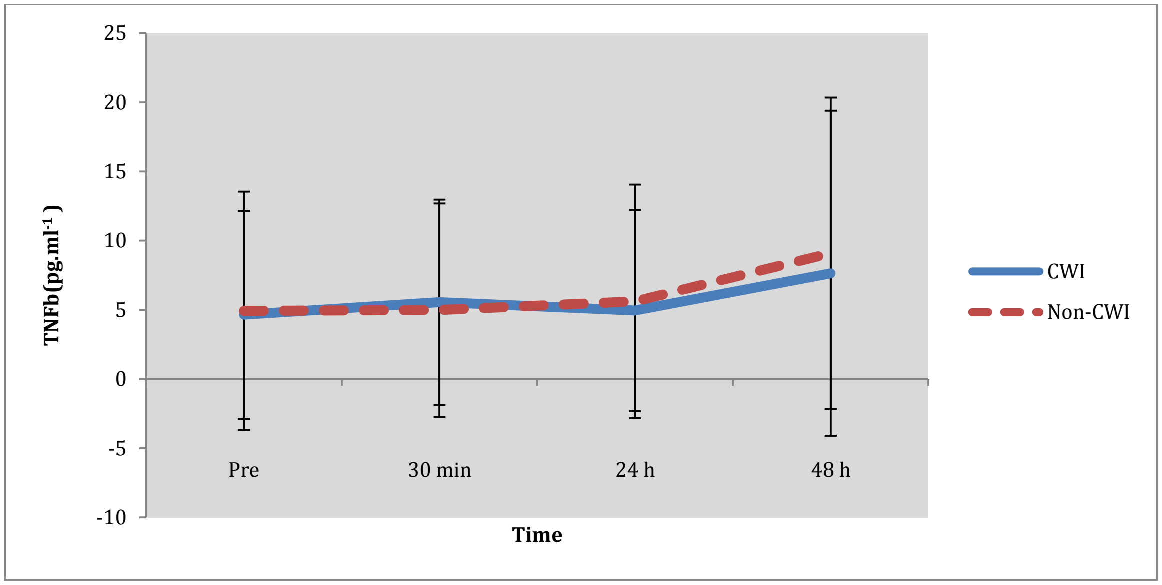


Figure 7. 25 Comparison between two groups for TNFb changes through the 48h recovery period.

7.3.5 Results of pro-Inflammatory Groups

Many of the cytokines showed a tendency to change over time, but the trends did not reach statistical significance, possibly due to low participant numbers giving low statistical power. Therefore, to increase statistical power, the clusters of pro- and anti-inflammatory cytokines were grouped accordingly and their changes over time were expressed as a %change from baseline.

Repeated measure ANOVA indicated significant differences between groups for the cluster of pro-inflammatory cytokines (p=0.023). Furthermore, there were significant differences over time (p=0.042). The level of pro-inflammatory cytokines increased immediately after step-up exercise (p=0.001) and remained elevated 48h post intervention (p=0.019) in the control group.

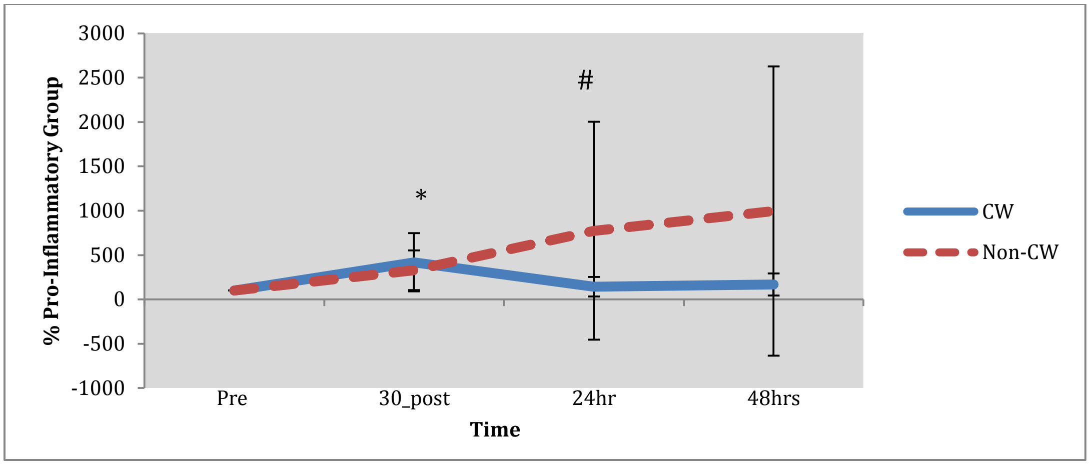


Figure 7. 26 Pro-inflammatory groups result of the cold and non-cold groups through the measurement period. (*) Indicates significant difference between groups (p=0.023), (#) Indicates significant difference over time (p=0.001)

7.3.6 Results of Anti-Inflammatory Groups

The results for clusters of anti-inflammatory cytokines showed significant differences between groups (p=0.012) during measurement time. The concentration of the cytokines was raised immediately post exercise in non-cold group (p=0.001) and stayed elevated 48h post intervention (p=0.024). There were no effects of time on the levels of anti-inflammatory in the cold-water group (p=0.063).

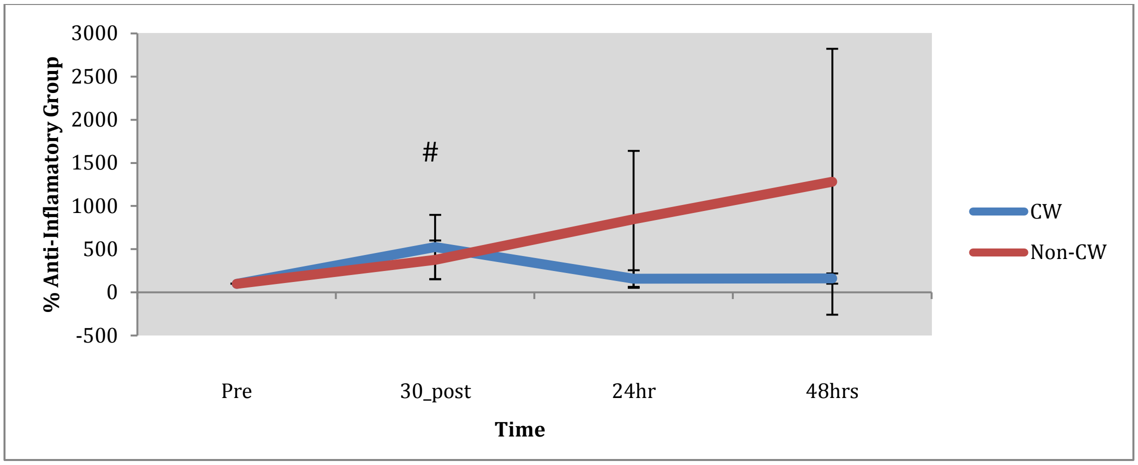


Figure 7. 27 Anti-inflammatory groups result of the two groups through the measurement period. (#) Indicates significant increase of the levels of anti-inflammatory in the non-cold water group (p=0.001).

7.3.7 Discussion

The levels of pro-inflammatory and anti-inflammatory cytokines, as well as stress hormones post-eccentric were examined before and after eccentric exercise and also comparing the effectiveness of CWI. In relation to anti-inflammatory cytokines, no significant differences were present in the level IL-10, IL-4 and IL-21 within plasma at any time point in either CWI or passive recovery conditions. A significant difference occurred in both IL-6 (p=0.013) and IL-5 (p=0.021) 24h post-intervention for both CWI and passive recovery conditions. The pro-inflammatory hormones INF**γ**, IL-17A, IL-9, IL-2, CCL20, TNFbeta concentrations peaked 48h post-intervention, and their levels may have continued to increase, but we were not able to take any values beyond 48 hr. Overall, no significant differences were detected between treatment groups for any cytokines or hormones.

Interleukin-6 has been used as a marker of exercise-induced stress (Pedersen, 2000), particularly with eccentric exercise (McKay et al., 2009; Nemet et al., 2009; Pournot et al., 2010; Lee et al., 2012; Nieman et al., 2012) and is also a sensitive marker of acute inflammation (Tomiya et al., 2004). There is variation in the reported peak level of IL-6 concentration from approximately 1.5 hours post eccentric exercise (Pedersen, 2000), to approximately 6 hours post-exercise (Willoughby et al. 2003) and 8–12 hours post-exercise, and concentrations return to baseline levels approximately 24h post-exercise in the absence of DOMS (Tomiya et al. 2004). The current study demonstrated an increase in the circulating concentration of IL-6 and IL5 within both groups 48 hr after exercise.

No effect to the level of IL-6 plasma concentration was observed at 1 hour post-exercise by Nemet et al. (2009) following a 250-meter intermittent sprint that was performed between ice application and a passive recovery group, although the concentration levels were not measured beyond 1 hour, which ultimately made it difficult to generate comparisons that exceed that time measurement. Thus, the current study expanded upon the timeframe in order to determine the measurements up to 48h post-exercise, which defined that the effects became evident post-24h. The majority of previous investigations have suggested that the reduction of the inflammatory response in the 24-h period following eccentric exercise is not effected by CWI (Rowsell et al., 2009; Halson et al., 2012; Lee et al., 2012; Chow et al., 2015). Lee et al. (2012) compared the responses of IL-6 post-90 minutes of treadmill exercise, as well as the recovery responses that measured the core temperature returning to 38.0°C following both 11.7°C and 23.5°C water (no passive condition). A decrease was observed in serum IL-6 concentration post-CWI in 23.5°C (69.8 ± 15.2%), whilst a minimal elevation was demonstrated at 11.7°C (30.5 ± 70.0%). However, it was challenging to determine the exact differentiations that could compare with a non-immersion group, as no control condition was incorporated. The discrepancy in findings between studies is often from the methodological differences, measurement timings, as well as peak level of inflammatory markers.

The results indicate that compared with the control group, the CWI group showed greater rises in pro-inflammatory cytokines at 30 min after exercise, but not at 24hr and 48 hr (Fig 7.26). It is not possible to know which cell types were principally responsible for the elevated concentrations of cytokines after exercise, since the majority are released from multiple cell types (Smith et al., 2008). A recent review has denoted that when CWI was compared with alternative protocols of recovery, a variety of studies failed to ascertain marked differences in the inflammatory response (Chow et al., 2015). Shivering induced by cold exposure is part of the thermoregulatory stress, which causes involuntary and repetitive muscle contractions that help maintain thermal homeostasis through the production of heat (Gagnon et al., 2014) and this was expected to have led to additional cortisol release (Pääkkönen and Leppäluoto, 2002) (Pääkkönen and Leppäluoto, 2000), although our results show no differences between CWI and control conditions in corticol responses. Previous studies have shown that the level of circulating cortisol in response to CWI may either decrease, remain unchanged or produce marginal changes (Pääkkönen and Leppäluoto, 2000; Halson et al., 2008; Bleakley and Davison, 2009). Methodological differences between groups may have led to the differences in findings.

7.3.8 Limitation of the study

The timeframe of measurement duration in the study was up to 48h post immersion, so we were not able to determine any changes that occurred after this time. Further work should extend the follow-up time beyond 48 and even 72 hours post exercise, as previous studies (Lee et al., 2012; Brazaitis et al., 2014) have detected changes in the inflammatory markers post muscle damage at 72 hours.

7.3.9 Conclusion

In conclusion, some of the inflammatory markers of muscle damage and stress hormones significantly contrasted between pre- and post- exercise in both groups, which indicated that step-up/down exercise induced an acute inflammatory response, although no conclusive differences were presented between groups. However, to provide more in-depth findings, additional research is required with a greater sample size and a more extended relevant time-course framework, although the current study has defined that CWI positively assists recovery.

Chapter Eight

Phase 3: Athlete’s Perception of taking part in CWI after training

8.1 Introduction

The following chapter is split in to three stages to understand how athletes respond to the idea of undertaking CWI. Firstly, an understanding of the perceived effects both prior to and following CWI from athletes are documented. Secondly, the chapter describes the form of research methodology that has been used in order to explore an athlete’s perception and belief of participating in therapy. Thirdly, the chapter determines the perceived physiological effects directly after a routine training program from the players’ viewpoint.

Recovery is essential to the high-performing athlete in order to reduce fatigue and enhance subsequent performance, particularly as the demands on athletes has intensified (Bailey et al., 2007; Ingram et al., 2009). Adequate recovery has been shown to result in the restoration of physiological and psychological processes (Halson, 2013). Different researchers suggest that enhanced recovery enables athletes to train more, and thus improves their overall physiological fitness, which includes strength measured in isokinetic dynamometer, power measured in drop jump and performance measured by cycle sprint (Vaile et al., 2008; Buchheit et al., 2009; White et al., 2014). Nevertheless, the psychological response of an athlete is a major contributing factor in the overall performance improvement, although it has not been adequately investigated previously

(Stacey et al., 2010; Broatch et al., 2014). This is due to the fact outcome measurements have not been documented, which provide conclusive results, as the psychological effects have not been perceived to present the same level as physiological effects. It has been stated that the speed and effect of rehabilitation can be duly affected by the fear that is derived from over-contemplation into the outcome of an injury, which is the athletes' perceptions towards CWI becomes a requirement of investigation (Moran, 2004).

Athletes' belief of the efficacy in an intervention could influence any subsequent response. Psychological factors, in particular expectancy have been suggested to significantly contribute to the both positive and negative sports performance (Beedie and Foad, 2009). Several studies have specifically investigated the perception of the physiological effect of CWI by measuring pain or fatigue using a different subjective scale (Sellwood et al., 2007; Bailey et al., 2007; Vaile et al., 2008). However, with long seasons in professional competitions, players may develop psychological stresses when they harbour a negative perception of CWI, which may lead to poorer performances (Kellmann, 2002).

Overall, the positive effects of CWI on the recovery of muscle strength and power, or on muscle damage after severe muscle activity, have been demonstrated, even though the precise reasons for these due benefits have not been conclusively stipulated (Bailey et al., 2007). Therefore, the researcher felt a vital part of the study was to explore athletes' perceptions of the effect and the benefit of CWI after training, as their physiological recovery may be affected by their psychological response.

In order to support the theories of cold-water immersion therapy and its physiological effects that were ascertained in the previous chapter, Phase 2 was implemented together with Phase 3 to instigate a mixed methods study. This comprised of both quantitative and qualitative research methods, which generated a base for richer data required, in order to comprehend the study collectively (Johnson and Christensen, 2008). Moreover, the entirety of the mixed methods approach was investigated through the requisite principal key areas of the CWI research; namely the physiological and psychological impact, as mentioned previously, which are central to the researchers' objectives in obtaining meaning and conclusions from the participants' personal experiences (DeMarrais, 2004).

Phase 3 of this research involved two principal methods of data collection that were planned by the researcher. The first was a questionnaire that was distributed immediately following the post-intervention session (appendix 8) and also during the measurement stage (Appendix 9,10). This was initially organised for the purpose of investigating the topic of cold-water therapy and also to generate new ideas, from which an interview guide could be developed for use in the following focus group. Then, after the completion of the intervention session and measurement stage, a focus group was interviewed for the purpose of gaining deeper insight and understanding of the participants' perceptions of the effect of CW and its subsequent healing attributes. This was to further comprehend the individual experiences and opinions of CW as a form of treatment. Hence, the data generated from the focus group could then be analysed after the session, along with the findings from the individual responses obtained from each person's three questionnaires.

8.2 Topic Overview - Justification of the methodology

Mixed method research entails the application of two or more sources of data or research methods in the investigation of a research question (Bryman, 2012). Thus, each type of data analysis becomes more enhanced by the other (Creswell, 2013). Through the use of a multi method approach for this study, the researcher initially implemented three self-completion questionnaires to gain a wider insight into the concerns and ideas regarding the perceptions of the athletes towards cold-water immersion therapy, followed by a focus group interview.

The reason for undertaking a qualitative approach in one's research is to understand more when little is known about the phenomenon under investigation (Patton, 2005). The majority of previous researchers investigated the physiological responses through measuring the inflammatory marker and evaluating the muscle performance. However, an athlete's belief and attitude to the efficacy of the CWI, their opinion of self-performance and their preference of CW application has received less attention by the researchers. In fact, there is limited comprehension of athletes' perceptions of CWI through existing CW research (Rowsell et al., 2011). Consequently, the objective of this study is to address this omission by previous investigations, as an athlete's psychological response and belief to CWI becomes fundamental to their overall physiological improvement. Therefore, the mixed-method approach addresses the necessary knowledge gap as to whether an athlete believes in CWI or not, as well as their reasons for their subsequent perception.

The researcher employed explanatory sequential design (Ivankova et al., 2006). An analysis of the three questionnaires was used to provide data from which an interview guide could be developed to use in the focus group. The focus group provided the researcher with the opportunity to explore these questions in greater depth and moreover, to investigate new thoughts that could not be covered from the questionnaire, such as how an athlete's peers may influence their perception. Furthermore, this type of multi-methods strategy enables the researcher to collect different types of data and provides an additional opportunity to discuss the CW phenomenon from various perspectives, in order to draw a variant and clear understanding of the data of opinions (Saunders et al., 2011).

8.3 Questionnaires

Questionnaires offer an objective means of collecting information about people's knowledge, beliefs, attitudes, and behaviour (Boynton and Greenhalgh, 2004). Questionnaire sometimes will be appropriate if used to quantify the finding of an initial exploratory phase (Gillham, 2008). Thus, the researcher wished to gain a deeper insight into some of the issues that were and were not identified in the literature, such as the effect of the CWI after training from an athlete's viewpoint. Subsequently, three self-completion questionnaires were conducted to provide quantitative data on CWI and to address the stated objectives below. Likewise, there are certain advantages to using a self-completion questionnaire: it is cost effective to administer, and can increase the reliability of responses due to the absence of the interviewer, which provides greater anonymity for the respondent (Seale, 2004; Gillham, 2008).

8.3.1 Objectives of the Questionnaires

The questionnaires used in this study addressed four specific objectives, as there has been a distinct lack of published prior knowledge:

1. To investigate the topic of cold-water immersion from the athletes' viewpoint.
2. To explore athletes' desire of using CWI after training.
3. To understand the reason behind choosing CWI as a recovery modality.
4. To gain a wider insight into the attitudes regarding the perceptions of the athletes towards cold-water immersion therapy (CWI).

The purpose of the questionnaires was to fill in the gaps, where necessary, of vacant information in relation to the literature and to further clarify the existing ideas and concepts gained from the literature review in an effort to strengthen the findings from this study (Cooper and Schindler, 2003; Blumberg et al., 2011; Hair et al., 2011). In fact, there has been no previous study that has highlighted whether an athlete's choice of CWI as an intervention modality was based on their individual belief of the benefits, or whether it was instilled from a practitioner's belief in the physiological impact.

Using self-completion questionnaires also reduces bias caused by the characteristics of the interviewer and the variability in interviewers' skills (Seale, 2004). The positive outcomes gained from the completion of the questionnaires can now justify the mixed-method set-up, as the valuable information that was collected has shown that players express their feelings or beliefs more freely after CWI, and provide significant amounts of data for further analysis (Hair et al., 2011).

8.3.2 Developing the questionnaire

The researcher aimed to extract the type of necessary question used in the self-completion questionnaires from a critical analysis of previous study literature. However, due to narrow information in the literature review, the researcher collaborated with supervisors PG and GY and designed questions to meet the purpose of the research, developing a series of specific questions that aligned with the objectives of the study. The researcher tried to avoid ambiguous and double paralleled questions during the process. Furthermore, the researcher considered the length of the questionnaires, by ensuring that they were not too long and that the questions were not overly complicated for the participants.

Three self-completion questionnaires were administered, and two of which dealt with: 1. The participant's feedback relating to the psychological experience of administrating CW (questionnaire 1), and 2. The participant's perception of the physiological effects of CWI in muscle strength and performance (questionnaire 2), which were both administered immediately after the participant had undergone CWI (day 1). The third questionnaire (questionnaire 3) was administered on the last day of the test and attempted to investigate how the participants had perceived the overall experience of the CW after two days had elapsed (day 3). In addition, the first and second questionnaires consisted of both closed and open-ended questions, while the third comprised of purely open-ended questions. The full extent of the questionnaire details will be provided in the following sections

Originally, a pilot questionnaire was initially administered to provide feedback on how to fully structure the final questionnaire. Hence, the closed questions were shown to be relatively easy to answer, based on this feedback, and the fact that they did not take long to complete. However, these closed questions were useful for finding specific information and allowing for the same frame of reference for all participants when choosing the answer, which could add significant weight to the data validity (Babbie, 2013). Conversely, open questions are considered an invaluable tool when the researcher needs to delve into a particular topic and allow participants to answer without limitation (Polonsky and Waller, 2010). In the current study, open questions were used to gain an understanding of the individual's feelings and opinions, which encouraged the flow of information, both immediately after the ice bath and two days after the CWI, (Mason, 2002).

8.3.3 Piloting the questionnaire

Piloting is where the researcher carries out a test with a small number of volunteers to see if there are any aspects of the questionnaire that do not work (Arain et al, 2010). A pilot test was conducted to ensure the understandability, usability and clarity of the questionnaires (Arain et al., 2010). The researcher administered the questionnaires (questionnaire 1 and 2) to only one undergraduate student and one postgraduate student, as according to Gillham (2000) one or two people is adequate to test the questions. Then the responses were analysed by observing the volunteer hesitation of answering the question or by asking the meaning of a question (Boynton and Greenhalgh, 2004). Additionally, the researcher detailed and considered all the feedback to gain better clarity, and following this, the feedback was discussed with the supervisors PG and GY. Results from the pilot test helped with improving the final structure of the questionnaires (Rattray and Jones, 2007). As a result, some questions were eliminated and more relevant questions in relation to the objectives were added. The pilot also provided an opportunity for the researcher to investigate whether the questions adequately addressed the issues surrounding the players' feelings (Gratton and Jones, 2010). Consequently, it was determined that a new questionnaire was needed to understand the participants' feelings regarding the overall experience in the following days after the intervention. Accordingly, the researcher developed Questionniare 3; the Day 3 questionnaire to ensure complete data coverage of this issue, which was administered without piloting.

8.3.3.1 Questionnaire 1. Participant Psychological Feedback Questionnaire (Day one)

The rationale of questioning an individual immediately after completing the CW intervention session was to focus on factors that could affect an athlete's belief in regards to using an ice bath as a recovery modality. This questionnaire was completed directly after the 15minute CWI session and a subsequent blood test.

The questionnaire had three parts (Appendix 8). The first part examined participants' demographic information (eg: Name, sex, age), as demographic characteristic need to be evaluated to determine whether they are affecting the relationship you view, such as between an exposure and response. The second part focused on the type of physical activity and the level of exercise practiced by athletes, in order to understand the backgrounds involved. In the final part, using both closed and open questions, the questionnaire attempted to quantitatively examine athletes' opinions on the ice bath with regards to duration, temperature, position, and safety. This information is important to understand an athlete's preferable methods of applying CW and whether this could stimulate a negative attitude toward the intervention. Moreover, their expectation of the experience, both physically and emotionally was addressed in the final question in order to compare an athlete's opinion immediately after the CW against the third day of the whole experiment.

8.3.3.2 Questionnaire 2. Participants' Perception of the Physiological Effects of CWI Questionnaire (Day one)

The rationale of this questionnaire was to understand the players' thoughts and expectations in relation to the strength performance test, which took place following the ice bath (Appendix 9).

This questionnaire consisted of four main questions. Each question utilised both closed and open sub-questions. The first and second questions addressed the beliefs of the respondents towards doing better in a dynamic and static strength test after CWI and before performing the strength test. Following this, the researcher used the same wording from the first two questions to understand the participants' feelings toward their performance in the strength test. However, questions 3 and 4 were designed to assess participant experience after the CWI and during the test in contrast to before CWI. The researcher attempted to observe two issues: first, whether the participants perceive any changes in strength following the ice bath, and second, what the level of leg comfort was during the test.

This feedback, which was completed immediately after the athlete had finished the CWI session, aimed to help the researcher draw a clearer picture of the data when compared to the quantitative measures regarding the players' expectations with the outcomes (strength and performance). Thus, the data would then indicate whether the results actually corresponded with the participant's prior expectations.

8.3.3.3 Questionnaire 3. Day 3: Participants General Experience Form

The objectives of this questionnaire were to investigate the development of the players' feelings toward the experience of CWI, in order to ascertain a clear picture of their perception.

The questionnaire was divided into two parts (See Appendix 10): the first part for the cold water group, and the second part for the control group who had not undergone CWI. Additionally, the questions in both parts had the same content, as both consisted of four questions. The researcher tried from these questions to investigate the reaction of the athlete when they were informed of the recovery group that they had been assigned to (CWI therapy group or control group), and if their feelings changed after doing the exercise, as well as questions on their feelings towards the level of pain after the recovery method. Moreover, the questionnaire was distributed to the athletes by the researcher on the last day of the study, as this elapsed time period of 2 days following the initial questionnaire could present possible contrasting findings. Even though an athlete's perception and belief had already been stated through the two prior questionnaires, a developing process of expectation and opinion requires feedback on a more substantial timescale than from its immediate effect. Hence, the Day 3 Questionnaire truly develops the full comprehension into both an athlete's psychological and physiological concept of CWI.

8.3.4 Reliability

The most important aspects when designing a questionnaire are reliability and validity. A perfectly reliable questionnaire elicits consistent responses (Williams, 2014). Although it is difficult to develop, it is reasonable to design a questionnaire that approaches a consistent level of response (Adams and Cox, 2008). Certain precautionary steps can enhance reliability and validity (Pettersen et al., 2004) and should be considered by the researcher: before administering the questionnaire, people with diverse backgrounds and viewpoints should review it to find out if the questions are clear and easily understood, if each question is interpreted in the intended way, and finally, if each question relates to the study topic and objective.

Considering this principle, the researcher collaborated with two Saudi physiotherapist researcher students in order to brainstorm the ideas and how to address them when developing the questionnaires. Moreover, the researcher's supervisors (PG and GY) reviewed the questionnaires and check the clarity of the questions. Subsequently, a pilot study was conducted to improve the validity and reliability of the questionnaire.

8.4 Data Analysis

A descriptive research method was used for this study together (Gillham, 2008). Hence, participant responses were converted into a numerical form and quantitatively analysed through documenting the amount of positive and negative statements. Likewise, the description of data will be presented in terms of frequencies and percentage. The open question will analyse, as certain researchers consider the responses to the general open questions to have a feature of qualitative approach (O'Cathain and Thomas, 2004). Thus, the answers in all the open questions from the current study were classified into a few data related themes, which were chosen according to the response of athletes with similar answers. Three themes were obtained from the three questionnaires: attitude during immersion, expectation of the experience and outcomes belief. In addition, the researcher will combine and link the open question responses to ascertain a broader data analysis understanding in order to support the athletes' beliefs.

8.5 Results

The researcher believed that the most convenient way to view the data was through a three-step process. Firstly, an examination of satisfaction levels from participants, in relation to the mechanism of using the cold-water immersion in terms of: duration, degree, position, experience and subsequent expectation [Questionnaire 1]. Secondly, this was followed by analysing the anticipation of the participants in relation to muscle strength performance after a period of CWI treatment [Questionnaire 2]. Finally, an observation of the change in the athletes' beliefs 48 hours post CWI, and how they view the experience afterward [Questionnaire 3]. Therefore, the combination of the analytical steps helped provide conclusive results regarding the athletes' overall general experience of CWI.

8.6 Questionnaire 1

8.6.1 Duration and Temperature

In the present study, the athletes were immersed for 15 minutes in a temperature of 12-13°C. 75% (7/8) of them believed that the duration was sufficient while 25% (one) felt it was excessive. Nevertheless, all participants stated in the questionnaire that the temperature was “cold enough”.

8.6.2 Position and Safety

Athletes were seated with their legs out straight in front of them, leaning on a backrest with their arms rested on the side of the inflatable bath. All participants answered in the questionnaire that they felt comfortable in the seated position and felt safe immersed with the water up to their waist.

8.6.3 Previous and Current Experience

All the athletes stated that they had previously used CWI post training up to their chest level as per a request of the rugby team’s physiotherapist. Furthermore, each individual used cold-water baths post match and post training. Comments from one participant included:

“*ice baths for 4 minutes after training and rugby games*.”

When considering the experience from another viewpoint and specifically in relation to the emotional and physical experience immediately post CWI, some athletes’ felt physical shock, numbness and a level of pain annoyance. However, other individuals experienced a distinctively more positive sensation post CWI. Comments provided from participants included:

" *annoying pain more than physical pain*"

" [I] *felt* [my leg] *went numb and sore for the first minute, also quiet shocked when I first got in*"

" [I] *felt normal, felt it should be done to feel better*"

" *quiet nice to get in after exercise* "

8.6.4 Experience Expectation

Both positive and negative expectations had been experienced by athletes prior to CWI, as they anticipated its function.

" *I was looking forward to it as I was hot*"

Whilst others were concerned at the impact of the CW to the body or potential unpleasant feelings

" *I thought I wouldn't be able to walk*"

" *that it was going to be a horrible* [feeling]"

8.7 Questionnaire 2

8.7.1 Question 1 and 2 - Athletes' Predicted Muscle Strength Performance

87.5% 6/8 of the responses from the athletes showed that static and dynamic performance would be better following CWI.

The athletes revealed through question 1 and 2 that their performance after having CWI would be the same as pre-test or better. One individual stated that he felt:

"[I] *will do better*, [I] *feel a lot better physically – both local muscle and whole body*."

Another player confirmed that the performance would be:

"*same as the pre-test, but feel would perform less well if no CWI.*"

Participants attributed the reason for performance enhancement as follows: the ice bath made them feel relaxed, refreshed and a lot better. One athlete stated:

"[CWI] *made my leg more comfortable…can't feel any sign and symptoms at rest, feel like normal*".

Another agreed:

"*I felt better after the previous ice bath...and feel refreshed after CWI*".

On the other hand, another participant stated that the sense of relaxation after CWI was the reason for a decline in muscle performance. One stated:

"After CWI, [I] felt better in myself, but more relaxed, so didn't perform as well."

This indicates that this participant did not think he would perform as well after CWI.

8.7.2 Question 3 and 4 - Results during the Strength Test and after CWI

After the CWI, 75% (7/8) of the athletes felt decreased in performance, while 25% (one) felt no difference. Likewise, 75% (7/8) of the athletes believed that their thigh felt weaker after the CWI, while 25% (one) felt no difference.

The athletes generally stated that their thigh felt weaker during the strength test, as exercises had an impact on the muscles, but overall the majority stated that they physically felt better after the ice bath. One individual stated:

"[my] *leg [felt] weak due to exercise, and felt much better compared to two weeks ago* [when I used CWI]. *Without CWI I was struggling a bit. With CWI I felt normal".*

Another athlete affirmed:

"[I] *felt weaker due to exercise but felt stronger compared with no CWI."*

Another individual referred to the numbness of the cold water as the reason for performing worse. He said that he felt "numb, not as strong or quick", which implied that due to the numbness experienced with the cold water, this player didn't feel his leg was strong enough to perform as well as in the previous test. In contrast, only a few participants experienced no differences in the performance, as one stated, "[I] felt like I was doing the test just over again". Overall, the questionnaire ascertained both positive and negative feedback in relation to CWI.

Even though, participants actually perceived a decrease in performance during the strength test, half of them felt a reduction in pain and physical stress after CWI compared with no CWI. "[I] feel it [leg] would be more fatigued without CWI," one player stated. Another supported this sentiment by stating, " I felt fatigued quicker than pre-exercise," while a third confirmed, "[I] feel [I] didn't perform as well as pre-exercise, but feel better [physically] than I thought I would”.

8.7.3 Results of the Comfort Test

Part two of the results from the second questionnaire concerned the level of thigh comfort while performing during the strength test. 37.5% of the responses from the athletes emphasized a feeling of more comfort during the test as a result of the previous CWI treatment; while 37.5% 3/8 of the responses showed no difference and 25% demonstrated less comfort during the test.

In relation to the thigh during the strength test, it was concluded that cold water numbs the pain and the athletes generally feel relaxed after CWI. One individual confirmed:

“…it [CWI] *has numbed the pain”,* while another concurred, *“*[I] *felt more relaxed.”*

Nevertheless, other individuals from the overall analysed group attributed their experience of feeling less comfort during the strength test to the effects of exercise prior to the test, as CWI had no effect. One player asserted:

“[I] *felt weaker due to the exercise”*

Another described the thigh reaction from the exercise as:

“[it] *starts shaking, no control.”*

However, it was clear that even if an individual experienced less thigh comfort during the strength test due to prior exercise, the positive impact of the cold water was considerable when compared to participating in exercise without the intervention, as stated by a third player:

"[I] felt less comfortable due to exercise but better than with no CWI."

8.8 Overall Experience Expectation

8.8.1 Questionnaire 3. Day Three Findings

The researcher presented the results of this section of the questionnaire in two parts through the study of two groups (passive and cold-water recovery). These groups were split into the distinctions of cold-water treatment and no cold-water treatment, and then following a period of two weeks were reversed. Firstly, the researcher focused on the reaction, opinion and expectations of the participants who experienced one recovery method, as they were the first individuals to be tested in the study. Secondly, the researcher actively compared the reaction from the contrasting experiences of each CW participant from both groups after they had experienced the tests.

8.8.2 Reaction, Opinions and Expectations – Group 1 CWI

It is not conclusive that all of the athletes liked the thought of the ice bath following exercise. 75% of the participants in the CWI group were eager to be in the CW, while 25% showed an initial hesitation to be in the CW.

The uncertainty of a desire to be in the cold water was due to the apprehension of the cold, but it lasted for only a few seconds. One athlete asserted:

"…here we go, it's going to be cold…the coldness only lasts for a few second or so,"

However, certain others showed enthusiasm for the idea of being in cold water as one participant said:

"I wanted to go in the ice bath. So, I didn't mind."

Nonetheless, when the researcher examined the results from the second question, and attempted to understand whether the desire associated with the use of CWI changed after participants had exercised, the findings showed that the views of the 25% who had hesitated using an ice bath changed their opinions. Subsequently, they joined in the belief from the original group who had wanted CWI that the process was useful or helpful. One participant stated:

"Due to how hot I was I wanted to go in more",

This was followed by another individual, who remarked:

"I know the ice bath make[s] *them feel better so I wanted to go in."*

The researcher attempted, in the following two questions, to assess the impact of exercise on the leg and whether participants believed that the feeling would be different without cold-water immersion. Moreover, all the participants agreed on the existence of mild soreness, as one athlete remarked that without the cold water the soreness would be greater. One other participant assumed that without cold water:

"…I would have been more sore in my leg overall."

Another confirmed:

"I think it would have been hurting a little more."

8.8.3 Reaction and Opinions of Group 1 – Passive Recovery

Initially, 75% of the participants in the passive recovery group felt relieved not to be assigned to the cold water, though 25% wanted to undertake cold water immersion.

However, the feelings of those who were reluctant to undergo CWI lasted only for a short period, as following the exercise, all of the participants felt that there was a distinct need for the cold water, as it was their perception that it would make their muscles ache less. Moreover, all of the participants agreed that after 20 minutes of step-ups, their feeling of cold-water apprehension changed due to their desire to relieve the pain. One participant stated:

"Yes [I wanted CWI]*, because my leg was aching."*

Another asserted:

"Yes [I wanted CWI]*, because I wanted the pain to go."*

The level of soreness in the exercised leg of participants when asked 48 hours post exercise was that 75% determined that they were "quite sore", while 25% experienced no pain.
In addition, 75% of the participants believed that cold water could enhance the speed of

the recovery. One participant emphasised:

"I feel cold-water treatment does aid in recovery of the muscle after intense exercise."

Another agreed:

"…it [my leg] *would have recovered more quickly."*

Nevertheless, 25% of the passive recovery group had a different opinion of cold water, which was that cold water could lead to more soreness. One participant stated:

"…the people that went in [to the cold water] are all hurting and sore."

8.8.4 Comparison of the data of both groups of participants

Following two weeks after the initial group distinction, the CWI and passive participants were reversed, so the CWI group became passive and the passive were treated by CWI. Therefore, the researcher could compare the relative data of the diversity of the thoughts, as all the participants would have experience of cold water but through a different initial expectation.

The results from the passive recovery group in the post-two weeks demonstrated that the initial reaction of the participants was that 50% of them were disappointed that they were not going in the cold water again, while 25% were content that they had not been able to, and 25% were caught between two minds as to whether they had wanted to enter the water. Half of the passive participants, who had experienced CWI two weeks previously, expressed their desire to use the cold water from the start. Hence, the participants' awareness of the effectiveness of the cold water could have been decidedly more acute. One athlete stated:

“[I] *wish that I had got to go in*”

While another said:

“*I was disappointed because I wanted one*.”

Therefore, the preference for the cold water was clearly implied by both individuals. Furthermore, the participants’ need for the cold water was increased by the post step-ups exercise, as one player stated:

“*I still wanted to go in*.”

However, the same participant who was concerned about the coldness in the first group felt pleased not to have been subjected to the cold water and stated:

“[I’m] *glad* [I’m] *not going in*.”

Nevertheless, after the step-ups exercise, the feeling towards the cold water changed for a third participant who hoped to cool down, which led to his own active search for CW. He noted:

“[I] *wanted to be cooled down*.”

On the other hand, uncertain feelings led to confusion for other participants. One was asked about his feelings after he had been informed that he would not have to go into cold water. The participant said:

"*Happy but a bit gutted 'cause* [there was] *no refreshing bath to cool myself down*."

The participant admitted the need of the cold water for refreshment but not to help with soreness. The participant thought his leg would feel worse with the cold water. He recalled his previous feelings when assigned to the CW group two weeks previously and confirmed:

"*…I think my leg would have been worse* [with CWI] *due to how my leg was last time*."

However, 50% of the participants felt the cold water was ultimately positive and they believed that it could help with the pain and soreness. In relation to the utilization of the ice bath, one participant noted:

"I don't think my leg would have hurt as much [as compared to not having an ice bath]."

Another participant stated:

"*…no pain as I feel it* [CWI] *helps*"

Surprisingly, as 75% of the original passive recovery group stated that they had felt relieved not to be assigned to the cold water two weeks previously when they were changed to the CW group 50% of them became happy to go in the ice bath and 50% were unhappy as a first reaction.

As soon as the participants began the step-ups exercise, half began to understand the need for the ice bath, as they could sense the difference that it would make to the muscle. One participant emphasised the need for the ice bath:

"*because I was in pain*."

Another individual added:

"…*I was tired and it* [ice bath] *looked refreshing*."

Many participants remarked on perceived soreness levels after the ice bath, from distinguishing between "less sore" or "a little bit sore". Furthermore, other participants also agreed that their soreness would have been greater without CWI. For instance, one participant said:

"*I feel I would have ached more* [without CWI]."

8.9 Focus Group

8.9.1 Aim

The aim of the focus group was to further explore athletes' perceptions of cold-water immersion following exercise by expanding on the limited structure of the predetermined questionnaire through analyzing and comprehending the results from the unanticipated issues in relation to CWI that will adapt to a natural environment that are not purely perceived opinions.

8.9.2 Objectives

The objectives of the focus group research were:

- To generate an understanding of why the athletes use the cold water.
- To investigate whether they notice the difference of pain and strength before and after cold water.
- To evaluate how they both physically and psychologically react to cold as a recovery modality.

8.10 Introduction

The second method that the researcher utilised to explore the athlete's perception of using CWI after training, was a focus group interview.

The concept and intention of such focus group led activities derives from the idea that groups tend naturally to form a collective and focused opinion (Duggleby, 2005).
Focus group interviews are defined by (Zikmund et al., 2012:117) as following "an unstructured, free-flowing interview with a small group of people". Whilst (Powell and Single, 1996) defines focus groups as "a group of individuals selected and assembled by researchers to discuss and comment on, from personal experience, the topic that is the subject of the research". Though, focus groups mostly rely upon interaction within the group, where the topic at hand is supplied by the researcher (David L Morgan, 2013).

Here, the researcher understands the aims and objectives of holding a focus group and aims to generate this valuable information and encourage diverse responses that will provide greater empathy and insight into the typical behaviours and unique perceptions of the participants, in relation to the issues regarding the research (Hennink, 2007). Even though, it had not been possible to perfectly anticipate the results, the researcher did endeavour to sufficiently report how the participants perceived and showed their personal views regarding CWI's reactions in the body whether they happened to be positive or negative.

8.11 The Role of a Focus Group

Holding a focus group-led interview allows the researcher to gather information from a small group, whilst observing non-verbal behaviour, giving direction where appropriate and probing the responses to further unveil key information that may later prove invaluable to the research (Cavana et al., 2001). In recent years, focus group activities have risen in popularity, as a method of choice, as it enables a research development team to construct qualitative data collection in a flexible and economic environment (Sekaran, 2006; Zikmund et al., 2012).

(Krueger, 2009) suggest that holding a focus group should be considered by the researcher, when they wish to understand the ideas and feelings that the participants have towards something. Likewise, to understand differences in perspectives of groups of people and to discover factors that influence opinions and encourage ideas. Moreover, the development from the group will help shed light on quantitative data already collected. The current study seeks to explore the nature of player perceptions of CWI, and the use of a focus group format allows the researcher to create an environment where the player can share their thoughts and perspectives.

In order to justify using a focus group for this study, the researcher provides the following reasons, which endeavor to explain the very essence of the decision to hold a focus group, the benefits it provides and the necessary requirements that a focus group can fulfill (Liamputtong, 2011). Focus groups have been shown to be an effective tool in the understanding of meaningful personal experiences and interpreting the actions and reactions of a specific person, or group (Morgan, 2013). Moreover, they are instrumental in opening the gateway of opportunity to expand the consensus and to potentially unveil additional key data (Cooper and Schindler, 2003; Cavana et al., 2001). Both of these reasons have assisted the researcher in approaching the study from two angles. From one perspective, the researcher refers to the data collectively and from another perspective the researcher can then link the questionnaires data and merge both explorations, thereby predicting suitable and logical conclusive results (Morgan, 1993).

Participants taking part in a focus group should have a high level of homogeneity to provide an ideal platform for free-flowing interactive conversation to emerge (Cavana et al., 2001). As such, the participants' backgrounds play a crucial role for the selection process. Thus, six Welsh rugby players participated in this study, as they all have a number of things in common which can be incorporated into participating in a focus group.

Furthermore, it is recommended that between 6 to10 persons who share similar social and cultural backgrounds is the optimum number of participants to take part in a focus group (Hennink, 2007). This will provide a group large enough to accommodate wider range opinions and perspectives, while still being small enough to maintain crowd order and familiarity amongst the group (Krueger and Casey, 2000).

It is also suggested that larger group formations can pose a problem in tending to an individual's personal perceptions, or following up a potential lead from questioning (Krueger, 2009). It can hinder the full potential of time allocation for responses and any chances for persuasive probing and in turn, each participant can be made to feel outnumbered, overwhelmed or feel left out (Rabiee, 2004). Where there is no manageable scale, the participants can come to a decision to remain submissive throughout the discussion or indeed can example only selective responses that withhold from revealing personal experiences (Krueger, 2009).

Successful focus groups have usually lasted for a period of around 1 to 2 hours, depending on the degree of the discussion flow, the information required and the scale of the group (Morgan, 1997). There are, however, opportunities to continue the pending research at a later date, where the researcher may decide to organize a follow up session, or indeed may have already pre-planned a series of short meeting sessions according to the needs of the research (Flick, 2009). Nevertheless, in the current study the interview lasted for an hour and a half and terminated when there were no more information that could be gained from the group. Therefore, no further follow-up interviews were required.

8.12 The Role of a Moderator

An interviewer in the focus group who asks the questions is called a " moderator" (Howitt, 2010). The moderator plays a central role within a focus group, so the researcher has acknowledged that the person performing this role must evidence the skills required that would create an open environment of topical debate and be able to steer the direction of the questioning content of the conversation in a challenging, yet persuasive manner (Stewart et al., 2007). The role of the moderator is also to make sure that all participants are encouraged to interact so that each person can contribute a fair and sufficient response, which would encourage every participant to provide key data. Thus, the researcher, being able to extract specific and diverse responses, would find it possible to offer a wider range of meaningful data to the study (Krueger and Casey, 2000). In this case, as English is a second language of the researcher, the researcher worked with two moderators, PG and GY, who have essential background knowledge of CWI therapy and expertise in conducting focus group interviews. In addition, they cater to the specific diverse language skills and adequate experience of dialect, which is a requisite for the study.

8.13 Developing the interview guide

The interview guide was developed using the research objectives (see section 7.9.2), coupled with the information gathered from the three questionnaires. With the assistance of the moderators (PG and GY), the researcher then prepared a draft list of suitable questions (Appendix 15). The researcher discussed these questions with the moderators to thoroughly evaluate the properties that they may offer to the data and in gaining maximum interpretive and reliable responses from the focus group (Rabiee, 2004). The early developmental stages of constructing each question must address any issues surrounding data compromise or critique post data and therefore, the researcher then considers these flaws that may occur from lack of data and any missing gaps in the research (Krueger, 2009). However, the researcher also acknowledges that the questions must encourage the participants to feel comfortable, thus highlighting the need for the role of the moderator, who plays a key part in delivering the questions persuasively and creating an environment that feels both open and informal in nature (Krueger and Casey, 2000).

The researcher then piloted the interview guide by conducting 3 semi-structured interviews with volunteer participants, which were audio taped. These questions were designed to gain the participants' views, experiences and opinions on the overall benefits of cold water (See Appendix 16 for the questions). The advantage of a semi-structured interview, with these types of questions, is that it can reveal how the participants' wish to respond, as well as reducing mediator bias and increasing the reliability (Krueger, 2009). The use of a semi-structured interview permits the moderator to vary the questions depending on the response from the group and emphasizes that the researcher can get more considered response from this flexibility (Silverman, 2006). Furthermore, semi-structured interviews can provide the opportunity to probe and leads the discussion into areas not previously understood, which is considered to be a highly effective method of collecting data for the research purpose (Saunders et al., 2011). The pilot, therefore, was pivotal in refining the focus group questions, which aided in removing repetition, as well as providing a questionnaire that encompassed open questions and removed forced or leading questions. Thus, the pilot study led to several minor changes in the questionnaire format.

8.14 Establishing and conducting the focus group

The procedure for recruiting the participants for this study has been discussed in Chapter

Three. However, additional information, especially relating to the focus group, is necessary to be explained. Focus group participants should be people who are strategically selected (Powell and Single, 1996). Through this, each participant's ideas and beliefs can be shared both collectively and comparatively. All of which provides an interesting and informal natured activity that indirectly opens the discussion wider, and in turn amplifies the level of pre-expected data extraction (Morgan, 1997). In fact, the participants in the focus group were drawn from the volunteer group following on from Phase 2 of this research (see section 4.3.2.2)

8.15 The role of the researcher

During the first of a total of three days of experiments, the researcher informed the participants that a focus group interview would be held during the third and final day of testing. The researcher then ensured that the participants were aware of the meeting venue.

The researcher acknowledged the necessary planning and equipment required to conduct the interview, such as having a digital tape recorder. Silverman (1997: 203) emphasizes this by stating, "…tape-recorded data have intrinsic strengths in terms of accuracy". The researcher then explained the purpose of the interview and subsequently requested verbal permission from the prospective participants to be digitally recorded during the interview. Likewise, the agreed participants were reminded of the pre-signed consent form from the first day and notified that the focus group would be a subsequent part of the agreed participating study. Additionally, the researcher informed the participants that they could withdraw themselves and their data from the process at any time, without prejudice. Consequently, of the 8 potential participants who took part in the second phase of the study, six consented to take part in the focus group interview.

8.16 Data Quality

The researcher considered the following issues in order to ensure that quality data have been collected:

- Reliability of information
- Forms of bias

8.16.1 Reliability

Where data is considered as a valuable source of understanding, the researcher also acknowledged that the data that had been generated must be reliable and consistent. With this, the researcher conducted the research adhering to the methodology of thematic analysis (Howitt, 2010). Moreover, most approaches to qualitative data analysis involve the identification and coding of themes. Assessing the degree of which coders can agree on codes (Intracoder reliability) is a useful concept in structural research, which is characterized by applied, multidisciplinary, or team-based work (Hruschka et al., 2004).

Therefore, In order to improve the level of reliability through this study, the researcher used double coding when analysing the data. Two persons [NA & GY] analysed the data independently, without interacting with the other; NA & GY then discussed the results

(Boyatzis, 1998). They agreed that the proposed theme of methodology adhered to the understanding of the study in order to achieve a greater level of reliability in the analysis. Moreover, the researcher used a multi-methods practice by combining the focus group with the self-completion questionnaire (Patton, 2002). Furthermore, the responses to the questions asked in the focus group were supported by the responses from the questionnaire.

8.16.2 Interviewer and interviewee Bias

Bias in research methodology is referring to “the presence of systematic error in a study” (Gerhard, 2008: 2159). Hence, bias can affect the validity of the focus group study. In order to prevent bias, it becomes necessary for the interviewer to understand the source and nature of bias that can have an impact on the study and to comprehend the steps that can be taken to cope with these biases (Stewart et al., 2007). Therefore, the researcher employed the following strategies to avoid bias in the study:

- To be prepared for the interview and that the questions had been pretested before the documented interview.
- To acknowledge the negative outcomes of leading forced questioning, as well as demonstrating a negative manner or otherwise counter-productive responses towards the participants.
- To understand his/her role of providing a comfortable environment in preparation for the setting for the interview.

8.17 Interview Procedure

For the focus group interview itself, the agreed procedure was to introduce a moderator and an assistant moderator to the task of conducting the interview for this study.

Whereby, both the moderator and the assistant were to perform specific tasks appropriate to their roles. The task of the moderator was to direct the discussion and to maintain continuity to the general conversation. Simultaneously, the assistant moderator was responsible for the task of operating the audio equipment and to ask any additional questions that may be valuable for further investigation or explaining misunderstood questions, such as giving a short summary during the key points of the discussion (Kruger, 2009).

At the beginning of the interview, the moderators firstly introduced themselves and thanked the participants for their time and involvement, and then subsequently proceeded to explain the purpose of conducting the focus group interview. It was agreed that the moderator should implement the suggestions of (Krueger and Casey, 2000) in relation to the questioning methods for a focus group. Through this technique it is suggested to include an opening question, an introductory question, a transition question, a key question and an ending question.

The initial question asked to the participants regarded "the reason for taking part in the study". This was to investigate whether the participants had had an experience of using this kind of recovery modality and to give insight into their perception of CWI. Once the participants started to explain their reasons, and had finished, the moderator then posed various questions in a more specific way to get depth and additional perspectives from the other participants. These questions included more conclusive information regarding different minor topics, for instance, details on individual sensations in CWI, previous CWI usage and feelings of allocation to cold water or passive recovery (see Appendix 16)
Each individual question was then followed up with a brief sub-question in order to extract more specific responses from the group, i.e, "sorry…it sounds like you were looking forward to the ice bath because you were hot and sweaty during the exercise?". Following

this, during the halfway point of the interview, the assistant mediator then proceeded to add further questions.

In addition, the moderators provided time to enable the participants to question the moderators themselves and/or to add more information if they liked. Once all of the questions and sub-questions were asked the moderators then completed the session. The digital recorder was stopped and the interview was then timely terminated. Overall, the time elapsed for conducting the focus group discussions was for the duration of an hour and a half.

8.18 Recording the data

Following the focus group interview, the researcher transcribed the digital recording verbatim. (Holloway and Wheeler, 2010) highlighted that the words of the participants should be preserved as accurately as possible by the researcher. Thus, the researcher should re-play the interview several times in order to ensure the adequate understanding of obtained data.

Due to the varying dialects of the interview participants and some language barriers of the researcher, this made the digestion and subsequent accurate transcription of the focus group interview more of a complex task for the researcher. Hence, the researcher proceeded to seek the assistance from an English Language teacher to perform the task of listening to the audio recordings taken from the interview. The involvement of the English Language teacher in the research was for the sole purpose of ensuring all the responses from the participants had been transcribed accurately and that the outcome of the prospective data would show perfectly accurate findings in the completed manuscript. In addition, the researcher advised all participants of the confidentiality relating to the data. Once the data transcription had been completed data analysis began.

8.19 Thematic Analysis Procedure.

Thematic analysis is a method for identifying, analysing and reporting patterns or themes, within data. It condenses and describes your data set in richer and more precise detail. However, it often goes further than this, and actually interprets various aspects of the research topic (Boyatzis, 1998).

After transcribing the interviews, the researcher became familiarised with the data following repetitive reading (Braun and Clarke, 2006). Thematic analysis was undertaken to determine if any common patterns emerged regarding athletes' perceptions of cold-water immersion following exercise using the following framework.

The researcher conducted the coding manually. Line-by-line, a search of transcripts was undertaken to highlight codes from the data. The researcher reached a count of more than a hundred codes, giving full and equal attention to the entire data. Coding the data pushes the researcher to engage with the collected material and to seek meaning, connections and insights (Polonsky and Waller, 2005). Subsequently, the transcript was reviewed once more by the researcher to check that common codes were actually prominent throughout. Then the number of those codes was reduced (known as the collapsing stage) according to the ones that have a similar meaning (Burnard, 1991).

To ensure the credibility of the findings the transcript was read by a supervisor (GY). The supervisor independently read the transcript and coded it using the approach described above. Following this, a meeting was then arranged to discuss the codes and to examine the level of agreement. Overall, a high level of agreement was achieved, although certain differences were initially discussed in order to reach a further joint consensus.

Once the researcher and supervisor had initially coded that data, the researcher began to analyse the codes, this included repeated ideas or statements (Burnard, 1991), and considered how different codes may combine to form categories and themes. The researcher then examined the relationship between codes, categories and themes (See appendix17). Furthermore, this process was accompanied by taking notes in regards to each code, and using different colours for varied themes.

8.20 Findings

The findings were generated from the focus group, which was undertaken to further investigate an athlete's perception of using CWI after training. From the focus group interview, four major themes emerged relating to CWI. These themes were" 'Awareness of the Benefit of CWI', 'Motivation for re-using CWI', 'Response to CWI' and 'Preference of using CWI'. The findings of each theme are explored and detailed below.

8.20.1 Awareness of the benefit of CWI

Through this theme the researcher attempted to explore how athletes understand and interpret the response of the body that is produced after training. The awareness of physical symptoms became evident after the athletes had each mentioned their own bodily responses after the exercise, though they did express that it was actually dependent on the type of exercise that they had undertaken. Following this, the athletes then considered their options for relief, and one participant stated for instance that:

> "*Doing my step ups, after about 5 minutes, I'm like…I really need that ice pack "cause my legs are gonna be hurting after a day or so.*"

Another individual then expressed a similar feeling about the same type of exercise, as he implied that the soreness was in the same area as the previous athlete:

> "*As soon as I started with my step ups, about 10 minutes in I felt a bit gutted that I wasn't [*had not used the ice bath*] 'cause I felt tired and sore already*."

Similarly, another participant described that the specific exercise that he undertook caused a lot of soreness:

> "*Especially if you did a 'legs' session your legs would be in absolute agony*."

One participant also reaffirms this; he cited that he experienced previous soreness while playing and added:

> "*'Cause I work on the front row, so I just get a lot of pain and my neck kills! I'm like this…I try to move my neck and it just hurts*."

It is possible at this stage to observe a distinct pattern that emerges from the participants that shows an awareness of their symptoms. They all express personal knowledge to which level of fatigue is most acute to them, which is perhaps in order to estimate the degree or type of pain they will have. One participant then went on to explain that the level of soreness could be somehow predicted, hypothesising that:

> "*Once you know the fatigue level that you're going through, when you're going through the five minute and the ten minute mark, you're like…it's gonna hurt and that's when you sort of think…that I need it* [CWI]. *So that might be an indicator*."

During the interviews, participants stated their own awareness to the level of exercise and further determined the types of exercise that could be the cause of their soreness during the training sessions. Each athlete expressed similar awareness and experiences, although they each mostly used a different terminology to describe the exercise that actually affected them. One did cite the general program they typically perform during training:

> "*…first, like fitness and weights*."

Another individual referred to this with more specificity:

> "*Yeah, if it was a skills session, then yeah* [I would not experience pain]*, but if they gave us a heavy set with more weights in the morning…*"

Where, a third participant could confirm the level of demand, regarding the exercise describing his set as a:

"*Heavy session*".

However, the fourth participant was more descriptive and could specify the kind of the exercise that they undertake:

"*So…leg presses, squats, pushing sleds*."

With the fifth player finally adding:

"*Pulling sleds*".

8.20.2 Motivation for re-using CWI

Individual motivation is vital to comprehend the reasons that result in a personal action, as the influence of an individual's needs and desires have a strong impact on the direction of their behavior (Brunstein and Maier, 2005). Hence, there is a need to introduce the theme of motivation in relation to reapplying oneself to the use of an ice bath. All of the participating athletes had had previous experience with ice bath therapy and their direct experience of its physical benefits was what personally motivated them to reuse it. Moreover, it is necessary to understand the origin of the athletes' own self-motivation. There were three categories of motivation that could summarise why cold water or ice baths were used for therapy: 1. Previous Experience, 2. Knowledge of Benefits and 3. Peer Influence.

8.20.2.1 Previous experience of Ice Baths

The majority of the athletes highlighted that they had previously used ice baths following training sessions. One player said that he had used cold therapy before and he felt better, mentioning:

> "*We do a day in* [a training session that incorporates CWI], *as you see, and it works out for me*."

Another had positive experiences with cold treatments in the past and felt it would be helpful now as well:

> "*When I've done it in the past* [the ice bath]*, I know it will be quite productive for me.*"

A third athlete confirmed the previous use of cold water immersion:

> "*Yeah, if I had it, say if we were away on our camp's training early morning and we had an ice bath…*"

8.20.2.2 Knowledge of the Benefits of the Ice Bath

The participants discussed the benefits of experiencing ice bath treatment post workout and its importance to the athletes' performance, providing them with strong positive sensations. One individual went on to further explain this, in which he felt better immediately after an ice bath:

> "*To be honest…like, first time I did it; with the ice bath; it felt better straight away*."

Another considered it a relaxing and refreshing activity after exercise, indicating actual pleasure from sitting in it:

"*I like to cool myself down. It makes me feel better…Yeah 'cause I like, had the ice bath and I enjoyed sitting in it*."

Another stated that sitting in an ice bath was the best form of therapy:

"*So for me there's nothing better than just sitting in the ice bath*."

The same athlete explained the reason for their enjoyment by stating:

"*'Cause I do enjoy it 'cause it cools you down and you just sort of, like, get time to relax in there and I just enjoy that part as well*."

On the other hand, while some looked to the ice bath as a positive form of therapy, others did not view the ice bath as a pleasurable experience. One said it was a painful experience:

"I just don't like being in an ice bath. It's too cold and it hurts my toes."

A different participant said that he was quite reluctant to undertake ice bath therapy but would do it:

"Yeah, I'm not keen on going in it, but yeah!"

Nevertheless, another stated that he would avoid ice bath therapy if it were not necessary:

"*No, I just don't want to be cold if I don't have to be*."

It can be seen through this research that the level of enthusiasm for ice baths varied among the players. Some considered it not only as a necessary treatment, but one that is actually pleasurable, while others preferred to avoid it if possible even if they felt there were benefits.

8.20.2.3 Peer Influence regarding Ice Baths

It is commonly understood that peer groups can have a distinctive impact on their contemporaries, whether that be positive or negative. Peers can wittingly and unwittingly encourage others to change their attitudes or behaviour (Gardner and Steinberg, 2005). This is particularly evident throughout this study, as athletes who began with negative thoughts later developed more positive thinking.

One participant stated that certain individuals tried to dissuade others from utilising ice baths until they actually became aware of the need for it themselves:

> "*The people that weren't were, like, saying 'Ooh, look what you've got ahead of you!' and teasing them and …suddenly it changed and half way through, you wished that you would have had the ice then, and that's quite a change really*!"

Another participant indicated that ice baths are not an optional choice in the training place.

> "*Yeah, first straight and its… 'everybody in'* [the ice bath] *and you would say 'they're making us go in* [ice bath]!'"

A different athlete confirmed that this was standard practice in training:

> "*Yeah, the same after a match…they'd be waiting for us soon as we come into the changing rooms. It's in there* [the ice bath] *before we go in for a shower, like*."

Overall, there are three things that motivate athletes to use an ice bath as a therapeutic modality: previous experience, perception or knowledge of the benefits of this therapy and peer influence. All the athletes that were interviewed had previously experienced the use of ice therapy and had differences of opinion regarding the degree of pleasure that could come from such treatment. However, most agreed that this was a beneficial form of therapy and expressed the desire to use ice baths as part of their training.

8.20.3 Response to CWI

One of the most important themes identified in the data is the response to cold-water immersion, as it is the core focus of the study. The information found in the data addressed athlete expectation of the treatment toward pain, fatigue and strength.

Numerous participants in the focus group mentioned pain relief. One individual stated that ice baths clearly stop the physical sensation of pain:

> "*Well, it just stops the pain*."

Another agreed that ice baths are an effective treatment for pain relief:

> "*It sort of, like, gets rid of the pain in a way*."

A separate individual described the pain relief offered by ice baths in terms of stages:

> "*It helps with the initial pain*."

While another explained this with more detail:

> "*You feel good for about half a day and, obviously the pain comes back, but not near as bad as what is was…like on the third day and on like the fourth day, the pain had more or less gone, this time I feel better*."

This statement indicates that ice bath therapy is an effective way to treat pain, but takes time.

The second aspect of a participant's expectation that was addressed is fatigue. The benefit of the ice bath therapy appears to be that it is effective in the treatment of fatigue. In fact, the data shows not only that pain decreases for the athletes, but that fatigue levels actually change after the ice bath. One participant compared levels of fatigue experienced on days with and without the ice bath:

> "*The day after I don't really have the fatigue, which I will have if I didn't have the ice bath*."

Whereas, another individual explained how he knew that fatigue was diminished after an ice bath:

> "*I think it feels after….after we did the first test, well, you start to work your leg muscles again…that's why it's not bad*."

The main concern of the athletes is to have enough strength to carry on training. It is evident from the participants' reactions that ice baths had an effective impact on strength. The athletes stated the reasons for that sense of power is because the feeling of pain is less:

> "*Today I feel stronger than the initial testing anyway and it sort of just numbs that pain a bit when you go and I felt stronger yesterday*."

In addition, the athletes defined strength in different ways, such as the ability to cope with the training demands the next day or the ability to move around freely. One individual stated:

> "*Early morning and we had an ice bath…by afternoon if we have an ice bath that will help you to carry on training*"

Another confirmed:

> "*Straight after the ice bath I could have got out and done another session*."

Furthermore, ice baths made a difference to the athletes when it came to using their legs after a heavy training session:

> "*After the ice bath I felt refreshed and like I was ready to go again, I could walk down the stairs without any problem or anything like that and I feel like even the left leg felt more, straight though. That sort of stung me a little bit…the fact that it was more straight and the ice bath just helped to refresh it quite instantly*."

Another individual agreed:

> "*It gave me more time to walk with*."

A third participant had a similar view:

> "*You start to work your leg muscles again*."

Moreover, improvement also occurred in a general bodily sense and was not restricted to specific parts of the body. One individual responded to the question: "Is it better in the 'whole body' sense?" with the short response of "Yeah."

While it appears from the interview that athletes tended to agree with each other in their responses to the ice bath, the participants emphasised that each one of their responses to the benefits of the ice bath was according to their own individual experiences. One stated:

> "*The way that they feel physically, then it makes me think it's* [the experience] *an individual thing, not as a set in stone thing…I think for me personally it's how*

intense I need it…I have done that, myself, from a training session. I set my own ice bath."

Another participant said:

"*And…this time, honestly, it's* [the general experience] *been much better. I don't know if it's just me, or what*?"

According to the data, the athletes' expectation of the effectiveness from ice baths was matched by the actual results. Ice baths proved to be effective in decreasing pain initially and over the course of several days (Leeder et al., 2011). Moreover, the reduction of pain contributed to the reduction of fatigue. Additionally, the athlete's actual belief in the benefits of using the ice bath would have a positive psychological impact on increasing the participants' own strength (Broatch et al., 2014).

8.20.4 Preference of using CWI

The concluding theme of this study is 'Preference', referring to the preferences of individual athletes when it comes to the use of ice baths. Preference is divided into two sub-themes: 1. Concerns, and 2. Application Preferences; which refer to the concerns that players had about the consequences of not using ice baths and the ways in which players preferred to apply the use of ice baths.

8.20.4.1 Concerns

One concern was that ice baths needed to be implemented immediately after training for maximum benefit or recovery would be delayed:

"*I set my own ice bath up but because the recovery had already started, it was already sore. It helps a little bit but not as much or as beneficial as it would have been as it would have done straight after…'cause the recovery process had already started anyway*."

A second concern and a consequence of not receiving ice bath therapy is that an athlete would experience more pain. One stated:

"*I'd push myself to do it but it'd be a bit more painful*."

Another agreed:

"*It would be killing…to push yourself to do it* [exercise] *without ice bath therapy*."

The inability to gain benefits from ice baths, as well as the uncertainty regarding whether ice baths are the best form of treatment for light activity, are the third and fourth concerns of athletes. In both cases, the participants were open to the possibility of exploring alternative methods of treatment. One stated:

"You've got to try and test it and see if it works for you. If it doesn't…then try another method."

Another said:

"I don't know whether …which sort of treatment that I need. Whereas, taking on replacements might be an adequate one for a light session but for a heavy session maybe looking at doing the ice pack treatment as a recovery set, instead of the light sessions."

8.20.4.2 Application Preference

The athletes' preferences in the application of ice baths for treatment were somewhat different from what they experienced in the study. This was divided into two separate spheres of preference, which were between the duration period of the treatment and the level of exercise stress before the ice bath. In relation to the time scale of usage, the participants expressed the desire for colder ice baths for a shorter duration. One said:

> "*I think the colder one* [which is shorter] *works better for me*."

Another stated:

> "*Like the one we had here did help but the cold works a bit better.*"

In addition, the athletes also stated that the use of ice baths depended upon the type or level of exercise. In fact, the heavier the exercise, the longer the treatment required. One individual said:

> "*After every game if it's only a training session and it's only a skills session, just a bit of work, you probably wouldn't get it* [ice bath], *but most times if it's gym session*, *then yes*."

Another confirmed this by saying:

> "[Ice bath] *depends on how you do. If we do two hard gym sessions a week, you'd be having it twice a week and three with a game*."

8.21 The Story of the Responses

Each of the data presented throughout this study has revealed significant findings, though more interestingly, it is the observation that the information obtained in Phase 3, from the questionnaire and focus group is what connects the research concept together. Hence, this connection relates an underlying background story in regards to the athletes' responses to an ice bath as a form of treatment.

The story in this research is that eight participants, all of whom are Welsh rugby players, had previous experience of ice baths and each showed their own expectations of using the ice bath for recovery following training. In fact, these expectations can play an important and distinctive role in the outcomes post recovery (Stacey et al., 2010). It has been shown that even if an individual merely expects an intervention then it can have a positive effect and subsequently improve an athlete's performance (Broatch et al., 2014).

Nevertheless, the initial belief from some athletes was that they would experience a horrible feeling of discomfort and also that the cold-water could affect the physical movement, such as the ability to walk post application. Furthermore, peer influence played a role in discouraging the participants to apply the ice bath directly after training, until the individual felt their own desire to do this themselves. However, after performing a mere 20 minutes of intense step up exercise the reaction of athletes regarding the cold-water altered as the need for the refreshment and for pain relief increased. Therefore, it is demonstrated that a strong belief in CWI, combined with any potential physiological benefits, will maximize its worth in recovery from exercise (Broatch et al., 2014)

Ice baths were used in this research process when the first sign and symptoms of physical stress appeared in the body of a participant after heavy training. This coincided with and concluded the search for the most beneficial method of recovery in order to resolve an athlete's pain and soreness so that the athletes could gain the strength to carry on training. Hence, one can observe that CWI has been shown to enhance feelings of recovery (Stacy et al., 2010) and reduce the perception of fatigue (Bastos et al., 2012) and muscle soreness (Ascensao et al., 2011).

The key motivation for the athletes in considering the ice bath as a recovery modality is that there is an evident desire for refreshment and cool down time after the sweat and exhaustion of exercise. Additionally, due to the level of refreshment provided by the ice bath post-exercise, the participants gained clear and distinctive awareness and acceptance of the proven benefits of such treatment in reducing pain and fatigue, as well as increasing strength. This has been demonstrated through a recent meta-analysis by (Leeder et al., 2011), that CWI is effective in reducing subjective measures of muscle soreness up to 96 hours post-exercise. Given the subjective nature of muscle soreness and the increasing popularity of CWI as a recovery strategy, it is increasingly plausible that athletes will believe and expect CWI to improve their recovery from exercise.

Following the comprehension of the application of the ice bath, the research methods were initiated in order to understand the perception from the select group of rugby players towards the use of the ice bath for recovery after exercise. Despite some concerns regarding the application of the ice bath and the use of it when it was perceived as too late in the recovery process, the need or desire by the athletes to use the ice bath after training was remarkably evident.

In further support of this, the overall belief in the effectiveness of CWI also significantly correlated to a greater recovery of strength, which demonstrated that a belief in the effectiveness of post-exercise recovery is directly associated with an individual's subsequent improved performance. Thus, by convincing an individual into thinking that they are receiving beneficial treatment (even if it is not proven) would instill a feeling of improved recovery and perceived readiness to perform. This is precisely the reason that this study measured both the physiological and psychological effects of using CWI, as both have been proven to play pivotal roles in the treatment process. In fact, a similar study tested the hypothesis that the acute benefits of CWI may at least be partly placebo related, and that altered perceptions of fatigue actually play an important role in recovery from exercise (Broatch et al., 2014).

Chapter Nine: General discussion and conclusion

9.1 Introduction

The current thesis has investigated three issues: firstly, to explore the physiological response of a healthy active body through CWI; secondly, to investigate the effectiveness of single application of CW following step-up exercise in reducing muscle soreness and minimising muscle strength decrements which accelerate recovery; and thirdly, to explore athletes' perceptions of taking part in CWI post-training. In the previous chapters (5,6 and 8) the findings of the investigation have been discussed. It was shown that clear physiological and psychological evidence regarding the effectiveness of CWI, as a recovery modality of DOMS post-training in elite sport, has not been fully elucidated. Furthermore, the physiological response of an active body at rest from cold-water immersion has not been fully understood Thus, this chapter will discuss the general results from the three completed investigations and their practical applications in order to ascertain a wider evaluative scope.

9.2 The acute physiological response of active healthy males to CWI

The validity of CWI as an enhancement method of recovery is assisted by determining and comprehending the reaction of the human body to CWI at rest (Halson et al., 2008). Specifically, during immersion, hydrostatic pressure and low water temperature play an effective role in the recovery of athletes, post exercise (Torres-Ronda and Schelling, 2014; Murray and Cardinale, 2015). Additionally, the consumption of oxygen and local vasoconstriction increases through a direct result of maintaining the core body temperature from CWI (Wilcook et al., 2006). However, the current study did not show any alteration of the physiological response to the body at rest that can be attributed to these factors. It has been suggested that different hormonal, metabolic and physiological responses of the body occur as a result of certain effects caused by CWI (Halson et al., 2008). Alterations in the cardiovascular system have been the most investigated beneficial effectual response caused by CWI, as a rise in arterial blood pressure, as well as peripheral resistance reductions in heart rate and cardiac output, have been previously documented (Wilcock et al., 2006). Likewise, CWI has been shown to reduce evident muscle inflammation, as a result of limited fluid into the interstitial space, which can be caused by the vasoconstriction of peripheral vessels (Halson et al., 2008).

The current thesis has demonstrated that CWI at 12-13°C for 15 minutes results in no significant changes in cardiovascular function, oxygen consumption and muscle strength in active healthy males at rest. However, the study has attempted to understand the relationship between the uses of CWI and the physiological changes of the body at rest. Consequently, the possible mechanisms that are responsible for the use of CWI in alleviating inflammation in sport environments and that improve strength can be evaluated and understood.

The typical cardiovascular response during the resting period in CWI is for the heart rate and arterial pressure to increase simultaneously (Wray et al., 2007). This occurs as cold-water temperature is likely to stimulate skin thermonociceptors, which leads to a reflexed increase in sympathetic nerve activity, and consequently reduces arterial flow (Gregson et al., 2011). In the current study, the results failed to observe marked changes in either heart rate or arterial pressure at 12-13°C, which is consistent with previous research by Gabrilsen et al. (1993). Contrastingly, HR and BP has been observed to increase in other studies that have utilised lower cold-water temperature, ranging between 1-8°C (Korhonen, 2006; Wray et al., 2007; Gregson et al., 2011). Hence, valid evidence has been produced that emphasises that colder water has a greater effect on the body (Dyckstra et al., 2009), whereas a different previous study shows that intra-muscular temperature of 7°C may not be sufficient to reduce blood flow and obtain desired physiological effects (Selkow et al., 2012). The temperature used in the current study was not cold enough to elicit physiological changes to the body, but it has been reported that a temperature of 15 degrees C may reduce intra-muscular temperature and cause physiological changes (Mueeusen and Lievens, 1986). Therefore, it is not possible to conclude that there is no effect of cold-water immersion on an individual's cardiovascular system under different circumstances.

In relation to oxygen consumption (VO_2), the results of the current study have shown that a slight increase occurs within 30 seconds of the immersion but it is not significant and the change persists for the initial three minutes, in spite of the observation by Sramek et al. (2000) that entering water had no effect on oxygen consumption. Similarly, previous research findings have also demonstrated that during head out immersion of most individuals the initial respiratory responses reach a peak within 30 seconds and adapt post-3 minute of immersion (Tipton et al., 2003; Datta and Tipton, 2006; Halson et al., 2008; Bleakley and Davidson 2009). The explanation for the initial increase in VO_2 could relate not to events initiated by the fall of skin temperature, but to events initiated by change in the ambient pressure acting upon the body when immersed in water (Mekjavic and Bligh, 1989).

In addition, decrease in muscle performance is strongly associated with decreasing muscle temperature (De Ruiter and De Haan. 2000). Skeletal muscle contraction is temperature sensitive, and parameters such as maximal isometric force production, the rates of force development and relaxation and maximal power production strongly depend on temperature (Ranatunga, 2010). The literature that examined the effects of CWI on muscle strength stipulated an increase in strength in certain instances (Vaile et al., 2008) and in some instance a decrease in muscle strength post-CWI (Mattacola et al., 2013). In fact, data from the present study did not show any change in muscle strength, as the muscle may not have reached the level of coldness that could elicit a measurable change in muscle strength.

Cold-water immersion has been shown to cause a slowing of motor nerve conduction as a possible effect of inefficiency of the musculotendinous unit (Mattacola et al 1993). Indeed, muscular contractile speed and force-generating ability may become decreased due to the reduced neural transmission (Wilcock et al., 2006). Nonetheless, many additional factors are prevalent that may alter the temperature and NCV relationship, as nerve depth, the subcutaneous tissue and variations in temperature can all contribute (Halder et al., 2014). However, the results from the current study exhibit no significant differences in KE MVC strength during the period of measurement, which coincides with the findings of a previous study (Leeder et al., 2015). Contrastingly, several studies reported a decrease in isometric strength post cold-water, although a full 20 minutes duration of immersion was implemented at different temperatures ranging from 10-15°C (Holewijn, 1992; Oksa, 2000;

Halder et al., 2014). Seemingly, the possible explanation for these converse results may be due to temperature and the duration of the immersion, as well as the morphological differences of the muscles that have been tested by other studies (Halder et al., 2015). In fact, it has been demonstrated that CWI is more effective in reducing nerve conduction parameters with more than 15 minutes cooling treatment (Rupp et al., 2012).

The rate of contraction on muscle contractile properties has been shown to be slower with lower temperatures, which results in less powerful contractions (De Rueitr, 2000). Hence, it has been postulated that less force is produced following cooling of the muscle (Racinais, 2010). Conversely, in the current study, no change in the muscle contractile properties post-CWI was noted, although a study found a decrease in twitch tension and force production when the triceps surae were electrically contracted following immersion of the leg in cold water, which was most pronounced at higher frequencies of stimulation (≥ 40 Hz) (Nolen et al., 2012). Moreover, they found that the rates of electrically elicited contraction force development were slower and that maximum twitch and isometric forces were significantly lower when tissues were cooled. Likewise, contractile force following cryotherapy was progressively depressed at stimulation frequencies (>50 Hz) (De Ruiter and De Haan. 2000).

In the current study, post-CWI force measurement was conducted an hour post- immersion with some participants, though not all participants were measured within the hour, and this may have impacted the results. Oliver et al. (1979) ascertained that at room temperature, 1 hour is enough to restore cooling induced decrement in muscle force, which may explain the result of this study. Additionally, the findings of the present study indicated that following 12-13°C a significant reduction on peak torque at movement velocity (300/s) was present. This supports the finding of Howard et al. (1994), which stipulate that the physiological mechanisms that limit high velocity muscle actions are not operational due to slower velocities of joint movement or isometric force production, which produce less effectual strength (Howard et al., 1994). Nevertheless, the findings of the current study are in contrast to the research by Haymes and Rider (1993), who found no significant decrease in torque at each of the three speeds 30, 180, and 300/sec.

A variety of factors contribute to the reduction in contraction speed, as changes in rate process with decreasing temperature may be a consequence of the effect of temperature on metabolic rate, and the maximal rate of adenosine triphosphate (ATP) hydrolysis (Sramek et al., 2000). There is also impairment of the calcium released from the sarcoplasmic reticulum (SR), reduced calcium sensitivity, and/ or impaired kinetic of the muscle fiber action potentials, which may all play a role on reducing contraction speed (Berchtold et al., 2000). Indeed, a singular or combination of these results may reduce the rate in the cross bridge cycle, and thus result in slowing the contraction velocity.

9.3 Effect of CWI on elite athletes' recovery

Cold-water immersion is a popular modality that is utilised by athletes to speed the recovery post intense exercise. Indeed, the most beneficial effect of CWI is to reduce soreness, to improve strength and to reduce inflammation in order to hasten recovery.

9.3.1 Effect of CWI on Perception of soreness

An intense bout of unaccustomed exercise that involves a significant eccentric component can lead to muscle soreness, which is known as DOMS (Eston et al., 2003). This is usually experienced within 0-2 hours post exercise, and becomes progressively diffuse by 24-48 hours post-exercise (Yanagisawa et al., 2004). Additionally, the soreness generally subsides by 5-7 days post-exercise (Proske et al., 2001). In general, pain, tenderness, stiffness and strength loss are the typical symptoms associated with DOMS (Connolly et al., 2003). Even though pain is a subjective sensation, its perception can inhibit performance among athletes (Snyder et al., 2011). Therefore, the reduction in soreness is essential to improve the performance in athletes (Day and Polen, 2010). Invariably, it has been shown that reducing the perception of pain associated with cold application is achieved through an anaesthetic effect (Burke et al., 2000), which is believed to relate to a reduction in nerve conduction velocity (NCV). However, in order to achieve cold-induced analgesia, the skin surface temperature must be lowered to approximately 13.6°C (Love et al., 2013). Skin temperature was not measured in the current study, so it is difficult to reach any conclusions as to the analgesic effect of CWI on soreness.

In addition, it has been noted in the current study that step-up exercise induces a considerable increase in perceived soreness immediately post-exercise; this was evident from the significant change over time in all of the dependent variables measured. However, by comparing the two-treatment groups, the results of the present study demonstrate that using the subjective pain measurement (VAS) scale does not indicate an altered perception of pain between the two groups during the time point. The findings are in accordance with others that have failed to demonstrate a beneficial effect of CWI in muscle soreness when using the same measurement tool (Jakeman et al., 2009; Corbett et al., 2012; White et al., 2014; Glasgow et al., 2014; Moreira et al., 2014; Leader et al., 2015). In contrast, previous studies, which used the VAS measurement, reported CWI to be better in reducing the perception of muscle soreness than passive recovery (Bailey et al., 2007; Ingram et al., 2009; Ascensão et al., 2011; Pointon et al., 2011). Different methods have been incorporated within these previous studies that could play a role in the results, such as an absence of a passive control group or applying the test on healthy subjects. Moreover, the subjective nature of pain is likely to account for some of the variability in the soreness measures. Furthermore, it has been reported in the review by Leeder et al. (2011) that more effective analgesic properties post-CWI appear following high-intensity, rather than through eccentric exercise.

Nonetheless, using a pain pressure threshold demonstrates a soreness increase in the passive group in the proximal muscle location that remains elevated during the recovery period, which correlates to previous studies (Eston and Peters, 1999; Brotach et al., 2014). The study by Eston and Peters (1999), measured tenderness at nine sites of quadriceps femoris and reported that tenderness at sites on the mid-belly of the muscle were less variable than at the proximal and distal musculotendinous junctions. However, even though the data highlighted that the passive group experienced more soreness in one site of measurement, no significant differences were noted between the treatment groups, which confirms the finding of Eston and Peters (1999).

9.3.2 Effect of CWI on muscle strength

The duration of time post-EIMD was observed to be a marked effectual factor in MVC decrement following damaging exercise protocols in both CWI and the passive group respectively ($p= 0.004$, $p=0.002$). There was a noted decrease immediately post-treatment, although no significant differences were noted between groups 24h or 48h post-intervention. The results of the current investigation support the findings that cold-water immersion has no effect on the recovery of knee extension maximal isometric voluntary contractions between groups (Sellwood et al., 2007; Jakeman et al., 2009; Corbett et al., 2012; Broatch et al, 2014; Glasgow et al., 2014). Nevertheless, other studies have shown that CWI is effective in returning the MVC strength to the baseline level following 48h post-immersion when compared to the control group (Vaile et al., 2008; Ingram et al., 2009; Pointon and Duffield, 2011). The reasons for these contrasting results may stem from differences in regards to the exercise models used (Pointon and Duffield, 2011), and variations in the immersion protocols employed in terms of duration and temperature (Bailey et al., 2007; Montgomery et al., 2008).

Conversely, a review by Leeder et al. (2011) concluded that CWI was effective in the recovery of muscle power and not in muscle strength after eccentric exercise, as type 2 fibres, which are the predominant fibre type in high-velocity muscle contractions that involve elevated power production, are preferentially damaged following eccentric exercise. Additionally, CWI is speculated as being associated with a heightened recovery of type 2 fibres, as following strenuous exercise such as various field sports, preferential recovery of power over strength may have implications for periodising training in subsequent days (Leeder et al., 2011).

Opinions similarly vary when describing the acute effect of cold on strength and velocity. Some authors state that exposure to cold can decrease dynamic contractile force immediately on application of a variety of cryotherapeutic modalities (Hadler et al., 2014).

Cold leads to an increase in the number of motor units recruited with each contraction and reduces the velocity of muscle contractions (Garcıa-Manso et al, 2011). In the current study participants experienced a considerable decrease in the muscle strength and muscle contractile properties immediately following exercise and 24h post-intervention. Thus, the results support the previous findings where force and power showed a decrease post-CWI during recovery periods (Crowe et al., 2007; Garcıa-Manso, 2011). Yet, even though, there was force decrement across time, there was no overall difference between groups across time. Therefore, these results suggest that CWI offers no functional benefit post EIMD.

9.3.3 Effect of CWI on muscle damage and inflammation

The principle hypothesis in the current study was that a single bout of CWI can be effective for modalities in reducing inflammatory markers and enhance recovery post muscle damage. The major finding was that some anti-inflammatory and pro-inflammatory markers have been elevated in both treatment groups over time, which suggests that inflammation was present as a response to damaging exercise. However, no significant therapeutic effect of CWI on any inflammatory cytokines compare to the passive recovery has been reported, although there was a different trend of CWI in some concentration inflammatory markers.

The levels of both IL-6 and IL-5 demonstrated significant differences across measurement time but no significant differences were observed between groups. However, the fact that both cytokines (IL-6 and IL-5) did not show any alternation in the concentration levels until 24h in the CW group provides a speculation that CWI may suppress the two cytokines, even when it is not evident. Comparable response was observed by previous studies that showed CWI did not influence IL-6 response of intermittent sprint exercise or cycling compare to passive recovery (Halson et al., 2008; White et al, 2014).

The level of cortisol decreases during the measurement time until 24h in both groups. However, despite the fact that the level of cortisol had the same trend in both group, the most beneficial effect of recovery session was recorded within 48h for the CW group, although it is not significant. It is therefore difficult to suggest with confidence that CWI improved cortisol levels post exercise. As a consequence, the result of a decreasing cortisol level match with the findings of Halson et al. (2008), who revealed that the cortisol level decreased for post-40 min cycling, which was followed by CWI.

In general, the results of the pro-inflammatory and ant-inflammatory denoted that the CWI group experienced a significant decline in the level of the cytokines at 24h post-intervention compared to the control group. In fact, a limited study supported the same results according to the review by Chow et al. (2015). Furthermore, efficacy is challenging to assess, although a range of immersion protocols have been utilised, and various markers of muscle damage have been measured through studies that have investigated cryotherapy as a recovery modality (Roberts et al., 2014).

9.4 The Athlete's Perception of Taking Part in Cold-Water Immersion post step-up/down exercise

The data that is presented throughout this study has revealed significant psychological findings from the questionnaire and focus group that can combine with previous physiological research concepts. Hence, this connection defines an underlying dual-experience in regards to the athletes' responses to CWI as a form of treatment.

The research focused on eight participating Welsh rugby players, who had experienced ice baths previously. Moreover, all the participants showed their own expectations of using the ice bath for recovery of soreness following training. Indeed, expectations can play an important and distinctive role in the outcomes post-recovery (Stacy et al., 2010). Hence, as pain is highly subjective in nature, the expectation becomes one of the psychological factors that can influence the pain (Atlas and Wager, 2012). It has been shown that even if an individual merely expects an intervention, then it can have a positive effect and subsequently improve an athlete's performance (Broatch et al., 2014). Furthermore, psychosocial factors can contribute to the belief in the symptoms of DOMS, which connect with the physiological factors in the pain experience (Nelson et al., 2013). Similarly, CWI can create a psychological sensation to awaken the body, which will ultimately reduce the effects of post-exercise fatigue (Glasgow, 2014).

Nevertheless, there was an initial belief from some athletes that they would experience a distinct feeling of discomfort to the cold water, which could affect their physical movement, such as the ability to walk post-application. Thus, belief, hope, expectancy, and other factors could have a stronger effect on recovery (Berdi et al, 2011). Likewise, peer influence played a role in discouraging the participants to apply CWI directly post-training, until the individual comprehended the benefit from personal use. After performing 20 minutes of intense step up exercise the reaction of athletes regarding the cold-water altered as the need for the refreshment and for pain relief increased. Therefore, it is demonstrated that a strong belief in CWI, combined with any potential physiological benefits, will maximise its worth in recovery from exercise (Broatch et al., 2014).

CWI was used in the current research process when the first sign and symptoms of physical stress appeared in the body of a participant following heavy training. This coincided with previous research that concluded CWI to be the most beneficial method of recovery in order to resolve an athlete's pain and soreness due to its analgesic effects (William et al., 2011). In this case CWI would aid the athlete to gain the strength to continue training (Rowsell et al., 2011). Hence, one can observe that CWI has been shown to enhance feelings of recovery (Stacy et al., 2010), reduce the perception of fatigue (Bastos et al., 2012) and reduce muscle soreness (Ascensao et al., 2011).

The key motivation for the athletes in considering CWI as a recovery modality is that there is an evident desire for refreshment and 'cool-down' time after the sweat and exhaustion of exercise. Additionally, due to the level of refreshment provided by CWI post-exercise, the participants gained clear and distinctive awareness and acceptance of the proven benefits of such treatment in reducing pain and fatigue, as well as increasing strength. Indeed, this has been demonstrated through a recent meta-analysis by Leeder et al. (2011), which evaluated CWI as effective in reducing subjective measures of muscle soreness up to 96 hours post-exercise. Consequently, given the subjective nature of muscle soreness and the increasing popularity of CWI as a recovery strategy, it is increasingly plausible that athletes will believe and expect CWI to improve their recovery from exercise.

Following the comprehension of the application of CWI, the research methods were initiated in order to understand the perception from the select group of rugby players towards this process for recovery post-exercise. Despite some concerns regarding the application of CWI, and the use of it when it was perceived as too late in the recovery process, the need or desire by the athletes to use it post-training was remarkably evident, which also corresponds to previous research that demonstrated greater benefits to be associated with immediate CWI (Williams et al., 2011).

In addition, the overall belief in the effectiveness of CWI significantly correlated to a greater recovery of strength, which demonstrated that a belief in the effectiveness of post-exercise recovery is directly associated with an individual's subsequent improvement in performance. Thus, by convincing an individual to think that they are receiving beneficial treatment (even if it is not proven) can actually instill a feeling of improved recovery and perceived readiness to perform (Broatch et al., 2014). Therefore, the current study evaluated the psychological effects of using CWI in order to combine with the previously researched physiological effects, as both play pivotal roles in the treatment process. In fact, a similar study tested the hypothesis that the acute benefits of CWI may at least be partly placebo related, and that altered perceptions of fatigue actually provide an important role in recovery from exercise (Broatch et al., 2014). This study highlighted that CWI improves the recovery perception post-exercise, which coincides with past research (Glasgow et al., 2014), and this will subsequently enhance performance (Cook & Beaven, 2013).

9.5 Strengths and weaknesses of the study

The current study is the first of its kind to investigate the physiological changes that an active individual at rest can experience when submerged in CW to the waist level, which has the intention to develop a founding base comprehension into different individuals' physiological responses to CW at rest. Most of the previous studies that have explored the responses and reactions from the body during the immersion to CW had incorporated a specific sport. There was no previous evidential literature from the studies in regards to CWI that investigated the wide range of the outcomes for the active individuals at rest. However, the extracted data from the current research may help to attribute to additional benefits in future studies. Therefore, through the inclusion of this new extracted data, the results into the effects of CWI have become enriched, which may add strength to the relevance of the present study, as well exhibit the potential gaps in the findings.

The current study recruited exclusively male participants, with no additional exceptions through the investigation. Therefore, the findings that were ascertained from each of the outcomes are primarily applicable to the male half of the population and cannot be applied to females. Thus, this is a potential weakness that may be adapted or enhanced in the future through structuring a study that involves the effects upon females from CWI.

Additionally, a separate weakness has been distinguished from the first phase of the present research that arose from the length of waiting period between CWI and blood measurements post-immersion in the active group, as the measurements were not taken directly following CWI, which may exhibit statistical significance and may decrease the opportunity to detect any alternations in the blood's beneficial hormone levels.

In relation to the second phase of the current study, which compared the CWI against the results from the control group, the overall structure is distinctly innovative. This is due to the use of VAS pain rating with a pressure pain threshold via algometer that functions to express a true indication of the severity of pain. These combinations of measurement may indicate more accurate pain ratings, as VAS and PPT represent different aspects of pain (Lau et al*.,* 2014).

Furthermore, the method that was employed in this study implemented step-up exercise for a period of 20 minutes that included wearing a weighted jacket. Consequently, this created a case of DOMS that were required to ensure that this form of exercise caused muscle damage in order to observe and calculate the effectiveness of the treatment. Most other studies utilised sport activities previously, which had involved eccentric and concentric movement (Ascensao et al., 2011; Elias et al., 2013; Peiffer et al., 2010). Indeed, only a few specific studies have used purely step-up exercise in the research process.

Another major advantage that has been shown by the current study has stemmed from the innovation in examining a wide range of outcome measurements that were tested in phase two. The study used a combination of the physiological and psychological alternations, while the focus group helped explore the psychological effects caused by CWI upon the athletes and obtained in-depth information relating to how athletes precisely perceive the benefits of CWI post-training. Moreover, the post-treatment questionnaire was incorporated in order to help identify any alterations that the participants may have made to their experienced pain and perceived performance. Invariably, there had been no literature previously that had used this combination of wide ranging data in the process of examination. Subsequently, the broader range of data, and especially in regards to the psychological aspects that has been provided by this study is helpful in providing a base for further study as a part of exploring the effectiveness of the treatment.

9.6 Limitations of the current study

9.6.1 Sample Size

The major principle limitation from the current study is in relation to the sample size, which was sufficient for measuring muscle strength, but low (n=8), as a more enhanced extensive sample would assist in determining statistically significant differences between interventions, especially regarding muscles' inflammatory markers, which were more diverse in findings. Invariably, a low sample number remains insufficient in the process of detecting minimal effects during measurement.

9.6.2 No direct temperature data

It can be seen through the present study that no direct intramuscular temperature data were recorded. Initially, the underlying assumption from the current study was that the effects of CWI are dependent upon the temperature that is induced by a given bath condition (duration and temperature). It was selected through this study to utilise CWI protocols with the expectation that they would elicit different intramuscular temperatures and thus, different therapeutic outcomes. Therefore, without direct intramuscular data, it remains impossible to infer any correlation between intramuscular temperature and recovery variables conclusively, as the findings merely create speculation into the reasons that one condition may be superior to another. In order to produce a fully structured and comprehensive conclusion for future use from the study, it would be necessary to ascertain a distinctive and definite correlation between one condition and the recovery variables, even though the current study has enhanced the possibility to hypothesise muscle temperature.

9.7 Key learning from the research

This study has shown:

- Physiologically, CWI does not improve strength quicker than passive recovery
- CWI prevents a reduction in the PPT 30 minuets afterwards resulting in athlete to feel less pain compared to passive recovery
- CWI does not affect inflammatory, stress, or hormone biomarkers compared to passive recovery
- Psychologically, some athletes benefit more from CWI because they believe it will improve their strength and reduce pain

9.8 Recommendations for further research

Research conducted for this thesis has explored the physiological impact of CWI on active healthy men and investigated the efficacy of CWI following intense exercise on elite athletes. Despite some encouraging findings throughout the current research regarding the efficacy of CWI post-exercise recovery, several questions surrounding water immersion appear to have remained unanswered. Hence, more in-depth information is certainly required within various potential areas of physical activity, so that CWI can be judged to be useful in unlimited circumstances. Therefore, further advanced research will be able to explore the effects of CWI in a wider range of areas, through the utilisation of a similar methodology from the present study.

9.8.1. Phase One: The physiological effect of CWI on elite athletes at rest

There is a concern that exists surrounding the body's response to cold-water at rest. Thus, the current research placed active healthy men for 15 minutes in cold-water immersion in order to understand how the body responds, both physically and physiologically, when immersed in cold-water. Nevertheless, it has now been acknowledged that the results may contrast when conducted in relation to elite athletes at rest, which needs to be further analysed in phase one. Similarly, further research is still required, in a standardised level of activity to determine the changes in skin and muscle temperature, as many of the physiological changes are suspected to be temperature dependent (White et al., 2014). It is possible that measurements thereafter might have shown an additional response that may be important to the interpretation of the effects of water immersion. Moreover, this would help to confirm any physiological and physical changes in the body that might result in more understanding of the body's reactions.

9.8.2 Phase Two: The effect of cold-water immersion on recovery of elite athletes

The current research has investigated the effect of 15 minutes of CWI on DOMS in elite male rugby players. However the effect of cold-water on soreness in women remains unexplored, which means that findings are questionable. This has left the topical analysis regarding whether women may experience more soreness than men post-exercise debatable and unidentified (Dannecker et al., 2012; Clarkson& Hubal, 2002; Dennecker, 2003).

Hence, a similar approach of methodology might be taken with elite female athletes in order to provide a better impression of overall results and allow a comparison between the findings.

Furthermore, it has been reported that anthropometric measurements of Saudi Arabian athletes were different than for athletes from western countries (Musaiger et al., 1994). Therefore, it would be conducive to an advanced understanding of the topic to use the same methods on Saudi athletes in order to compare the results and findings.

Several studies have provided indirect evidence that a bout of eccentric exercise in the arms compared with the legs can induce larger increases in creatine kinase activity in the blood, DOMS, as well as an elevated decrease in force (Paschalis et al., 2010; Nosaka & Newton., 2002; Dolezal et al., 2000; Nosaka et al., 1991). Therefore, research incorporating a variety of different types of exercise, which include both upper and lower eccentric exercise, could be explored to ascertain an in-depth evaluation of the overall effects.

The results from the current research have suggested that an athlete's preference in regards to CWI application is for a shorter and colder period of immersion. However, even though multiple immersions have previously been examined, the effective immersion protocol still remains unknown. Therefore, in order to manage the soreness associated with exercise effectively, future research will additionally explore and evaluate athletes' preferences, as well as the rationale for alternative cold-water immersion such as partial or whole body immersion.

Finally, field studies during competition would be the ideal research design, whereby the findings can be directly applied to the implication of real physical activity settings. Indeed, “new predictive data of DOMS in healthy individuals in work and sport settings could lead to better guidance, prevention, and treatment of DOMS” (Soer et al., 2009: 239).

9.9 Conclusion

Despite the study not producing the conclusion that had been initially anticipated, an abundance of beneficial concepts have emerged from the findings. The current study was initiated with the hypothesis that would be possible to ascertain a level of information from understanding the physiological changes, such as cardiovascular, respiratory and muscle strength that the body may experience when exposed to 12°-13°C of CWI for the duration of 15 minutes. However, the findings have suggested that following CWI, no effect on cardiovascular and muscle strength is shown, although this does not exclude the possible effect of cold-water therapy through different circumstances that have not been investigated during the present study.

The level of soreness that participants experienced post 30 min following step-up exercise, and the increased level of inflammatory cytokines, indicates that muscle damage occurs. This experimental study failed to demonstrate that a single bout of cold-water immersion at 12°-13°C for 15 minutes can be superior to normal passive recovery in preventing or reducing the intensity of DOMS symptoms, while improving strength. Nonetheless, potentially positive benefits are likely from the refreshing feeling of CWI, as well as the participant’s expectation, which may provide an important and distinctive role in the outcomes post-recovery.

Overall, the current study reinforces the importance of using CWI post-exercise, even though the complete level of benefits has not been comprehensively ascertained. It can be deduced that a short duration (5 minutes) with low temperature was the preference of the participants, as it has been perceived to help reduce the soreness and speed the recovery.

Conferences Posters participation

- Postgraduate Poster conference (2012)
- The 6th Saudi scientific international conference (2012)
- 2nd International congress on sports sciences research and technology support (2014)
- 9th Annual RIHSC Conference (2015)

9.10 References

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9.11 Appendix

Appendix 1: Faculty Etheical Approval

FACULTY OF SCIENCE AND ENGINEERING

M E M O R A N D U M

TO Jamie McPhee

FROM AnneMarie Walsh

DATE 24 October 2015

SUBJECT Faculty Ethics Committee Application **(SE111218)**

At the Faculty Ethics Committee held on Wednesday, 23rd May 2012 the Committee considered an application for Ethical Approval from Dr Jamie McPhee **(SE111218)** entitled "The Use of Cold Water Immersion to enhance post exercise recovery in sports"

The Committee requested modifications as detailed on the Scrutiny Report.

I am pleased to inform you that your recent resubmission has addressed these points satisfactorily and your application has received favourable opinion by Chairs Action

The Committee requires that you report any Adverse Event during this study immediately to the Chair and Committee Secretary. Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence (currently Prof Bill Gilmore).

Regards

AnneMarie Walsh
Research Degrees Group Officer
All Saints North

Appendix 2: Study Announcement

Manchester Metropolitan University

Faculty of Health & Psychology and Social Care

Study Participants Needed

We are seeking volunteers to participate in a research study designed to explore whether Cold Water Immersion speeds up recovery after exercise.

To be considered for the study, you must:

- Be between the ages of 18 -40 years old;
- Be willing to have a blood sample drawn
- Have no history of vascular, cardiovascular disease;
- Have no history neurological or skeletal muscle disorder
- Have no history of trauma, injury or cold insensitivity
- Active in any variety of sports

If you're interested in participating, please contact

Noorah Alshoweir at: 077 8701 4341

Email: nalshoweir@gmail.com

Appendix 3: Participants Information Sheet

Participant information sheet

The use of cold water immersion to enhance post-exercise recovery in sports

Study Background

Intense physical activity (exercise) can lead to muscle damage and muscle soreness. The soreness is experienced as a dull, aching sensation from the muscles that were used during the exercise and can last up to 48 hours after exercise was finished. Most people who are active in sports will be familiar with this sensation of aching legs and fatigue that comes after exercise. It is known as delayed onset muscle soreness (DOMS) and occurs due to the release of proteins and substances from the muscles that were slightly damaged during the exercise. The damaged muscles can take several days or sometimes a couple of weeks to recover. The muscle damage is actually an important part of the adaptation process because the subsequent repair makes the muscles stronger and more resistant to future damage. Nevertheless, athletes and sports clubs are always seeking ways to minimise the damage or to speed the recovery process. One strategy that has become very common in elite and sub-elite sports is cold water immersion. After intense exercise, athletes use cold water immersion (CWI) in temperatures between 10 – 15 degrees C for up to 15 min in the belief that muscle damage will be restricted and recovery enhanced so that subsequent athletic performance will be optimised.

However, there is not much scientific evidence to support this practice and we therefore have set out to investigate the physiological response to cold water immersion and establish whether cold water immersion really can reduce muscle damage and soreness to improve subsequent exercise performance.

Who can take part?

We are looking for young men and women aged between 18 and 40 yrs who are currently healthy and not suffering from neuromuscular, metabolic or cardiovascular disease. Participants will be required to abstain from alcohol, caffeine, and therapeutic treatment including anti-inflammatory drugs for the duration of the investigation. We will ask you to come to our research labs to complete 3 separate trials, each lasting 1 – 2 hours. The trials will be completed in random order and there will be at around 2 weeks break between trials. You are free to withdraw from the study at any time and do not have to give us a reason. We will be happy to answer any questions that you might have and we will only begin the tests when you are ready.

What is involved?

Trial 1

Following a 12-hour overnight fast, we will collect a blood sample and then use a magnetic resonance imaging scanner to measure the size of the muscles in your leg. You just need to lie down in the scanner and the machine will generate images of your leg. This takes about 20 minutes. We will then ask you to stand in a container for 15 minutes that has cold water (around 10 degrees) filled up to your waist. We will record your heart rate, breathing, blood pressure and metabolic rate while you stand in the container. We will ask you to return to the labs 24 hr after the exercise to provide a blood sample, test your strength and complete a quick assessment to tell us whether you are experiencing any muscle soreness.

Trial 2
We will measure your leg strength (just 1 leg) using a mixture of voluntary and electrically stimulated contractions on a specialised strength testing machine used by sports clubs and physiotherapists. After this you will perform 30 min of exercise stepping up and down on a step that is 30 cm high. We will ask you to return to the labs 24 and 48 hr after the exercise to provide a blood sample, test your strength and complete a quick assessment to tell us whether you are experiencing any muscle soreness.

Trial 3
This will be identical to trial 2, except the measurements will be performed on your other leg. The exercise will be immediately followed by immersion in cold water for up to 15 min. Immediately after the cold water immersion we will again test your leg strength. We will ask you to return to the labs 24 and 48 hr after the exercise to provide a blood sample, test your strength and complete a quick assessment to tell us whether you are experiencing any muscle soreness.

Are there any risks in taking part in the study?
The people who take part in this study will be familiar with exercise and probably also familiar to the sensations of aching legs the day after exercise. You might feel a little fatigued and possibly weaker, but this should not interfere with your usual daily activities. Some people find the electrical stimulation of the muscle to be unpleasant due to the muscles being contracted quickly without you having to activate your muscles voluntarily. However, this is a very quick and easy test involving less than 10 contractions and after some familiarisation at very low intensity, most people tolerate the stimulation without any problem. If anybody does not like the stimulation then we will not continue with it.
In taking part in this study it is likely that one or both of your legs might ache for one or two days afterwards. The reason is that whenever people do any heavy exercise, such as performing maximal strength contractions, the muscles become slightly damaged. The muscles then undergo a repair process and are subsequently stronger (this is the training-effect). This sensation of aching legs is felt most when you are sitting around and not very active, but when you begin to walk the legs will no longer ache. The sensation will last for around 2 days.

During all exercises and tests, and at the 24 and 48-hour follow-up, you will be monitored and questioned for signs of discomfort, injury or adverse reactions by a qualified physiotherapist.

Appendix 4: General Heath Questionnaire

General Health Questionnaire Date ....

name.. date of birth ....
Age................................ Gender................................

Home Address:

1 How would you describe your present level of fitness?
Sedentary Moderately active Active Highly active

2	Have you had to consult your doctor within the past 6 months? If you answered Yes, please give details...	Yes	No
3a	Are you presently taking any form of prescription medication? If you answered Yes, please give details...	Yes	No
3b	Are you taking nutritional supplements? (e.g. multivitamins, Vit C, cod liver oil) If you answered Yes, please give details...	Yes	No
4	Are you suffering from any known serious infection?	Yes	No
5	Have you had jaundice within the previous year?	Yes	No
6	Have you ever had any form of hepatitis?	Yes	No
7	Are you/might you be HIV antibody positive?	Yes	No
8	Have you ever been involved in intravenous drug use?	Yes	No
9	Are you haemophiliac?	Yes	No
10	Have you ever been diagnosed with any of the following:		
a	high blood pressure	yes	no
c	heart problems, heart attack or any heart complaint	yes	no
d	stroke	yes	no
e	diabetes	yes	no
f	lung disease/bronchitis/COPD	yes	no
g	asthma	yes	no
h	arthritis (osteoarthritis or rheumatoid)	yes	no
i	osteoporosis	yes	no
j	cancer	yes	no
k	parkinsons disease	yes	no
l	epilepsy	yes	no
m	anaemia	yes	No

If you answered Yes to any of the above, how long have you had the problem and do you still suffer from the problem? give details...
....

11 Do you currently have any muscle, bone or joint problems? Yes No
If you answered Yes, please give details……………………………………………………………………
……

12 Have you suspended your normal training or activity levels in the past 2 weeks? Yes No
If you answered Yes, please give details……………………………………………………………………
……

13 Have you broken/fractured a bone in the past 10 years? yes No
which bone or body part? ……………………………………………………………

14 Compared to others your own age, would you say that your physical health was:
much better, better, same, a bit worse, much worse

15 Compared to others your own age, would you say that your mental health was:
much better, better, same, a bit worse, much worse

16 Do you play sport or exercise? Yes No

If yes,
Which sport do you play most frequently? ………………………………………………………
How many hours per week? <1/ 1-2/ 2-4/3-4/>4

17 Have you participated in any form of exercise testing in the past? Yes No
If yes, was the test terminated prematurely for any reason? Yes No
If you answered Yes, please give details……………………………………………………………………
……

18 As far as you are aware, is there anything that might prevent you from completing the tests that have been outlined to you? Yes No
If you answered Yes, please give details……………………………………………………………………
……

Participant statement: As far as I am aware the information I have given is accurate

Signature……………………………………………………

Appendix 5: Participants Consent Form

Participant informed consent. ID code ……………………………

Name: ………………………………………………… Sex: Male / Female

Date of Birth: ………………………………… Age: …………

The use of cold water immersion to enhance post-exercise recovery in sports

Principal Investigator: Dr Peter Goodwin

Investigator/Collaborators: Jamie McPhee, Gill Yeowell, Norah Alshoweir

Ethics approval number: **(SE111218)**

I have read and understood the information sheet and all verbal explanation outlining the purpose of the study and the assessments involved. I understand that any tissue (blood) samples I donate will be stored in the university tissue bank with a unique identifier code and it will remain there until analyses are complete after which time it will be destroyed.
Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without having to give a reason. My concerns regarding this study have been answered and such further concerns as I have during the time of the study will be responded to. It has been made clear to me that, should I feel that my rights are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the Chair of the Ethics Committee of the School of Healthcare Sciences, Manchester Metropolitan University, Oxford Road, Manchester, M1 5GD.

Signed ______________________ Date ______________________

Name (Print)………………………………..

Witnessed ______________________ Date ______________________

Appendix 6: Rugby Player Instruction Sheet

CWIK Study

The use of cold water immersion to enhance post-exercise recovery in sports

Participant Instructions

How to get to the John Dalton Campus

John Dalton Building, Manchester Metropolitan University,
Oxford Road, Manchester M15 6BH

From the **M56**, south of Manchester, keep in right hand lane at Junction 3 (signposted City Centre) and continue from the end of the M56 onto A5103 (Princess Parkway), signposted Manchester City Centre. Continue ahead until you reach a major roundabout with an overpass and take the third exit, signposted Sheffield A57(M). At the next roundabout take the first exit onto Cambridge Street, turn immediately right onto Chester Street, the MMU car park is first on your right.

Satellite Navigation:
Input the postcode **M15 6BH** into your satellite navigation system to get to the All Saints campus.

The Car Park:
The car park is at the back of the building; at the barrier press the buzzer and inform Security you are visiting Nora Alshoweir.

Once in the car park, You will need to buzz for admittance – just say you're here for the CWI test. You must inform reception of your arrival and display a ticket in your car. A ticket has already been organised for you based on the information you provided.

Reception:
The John Dalton Building entrance and Reception is on Chester Street in front of Nando's Restaurant. There is a statue of John Dalton in front of the building. Once you have arrived at Reception and you have organised your parking, please call **Nora 07787014341**.

Who can take part in the study?

- Men between the age of 18-40
- Active at elite level in sport
- *Able to fast for 12 hours prior to each test*
- Willing to have a blood sample drawn
- Have no history of vascular, cardiovascular diseases, neurological or skeletal muscle disorder, trauma and injury or cold sensitivity
- Have no history of allergy to electrode adhesive

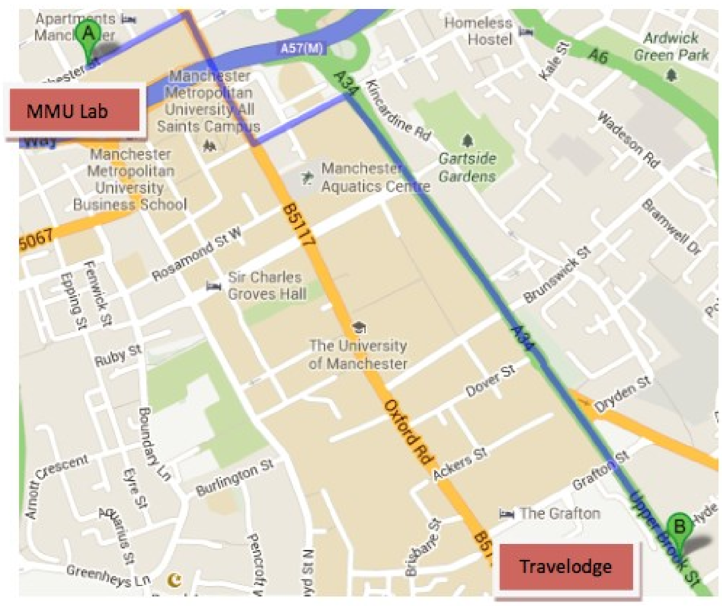
What do you need to bring with you?

- Towel
- Two swimming shorts

What will we provide?

- 3 free nights hotel stay a short walk from Manchester City Centre
- Meal voucher for the 3 days
- Free parking for the duration of the testing
- Data from the test

Accommodation:
Address: Travelodge, 227 Upper Brook St, Manchester, Greater Manchester M13 0HB
Phone: 0871 984 6487
Time taken to walk between the lab and hotel is 15 minutes



Important phone number:
Nora 07787 014 341

Continue Appendix 6

Important:
Please do not eat or drink 12 hours prior to your appointment

CWIK Study

The use of cold water immersion to enhance post-exercise recovery in sports

Participant information sheet

Study Background
Intense physical activity (exercise) can lead to muscle damage and muscle soreness. The soreness is experienced as a dull, aching sensation from the muscles that were used during the exercise and can last up to 48 hours after exercise was finished. Most people who are active in sports will be familiar with this sensation of aching legs and fatigue that comes after exercise. It is known as delayed onset muscle soreness (DOMS) and occurs due to the release of proteins and substances from the muscles that were slightly damaged during the exercise.

Damaged muscles can take several days or sometimes a couple of weeks to recover. The muscle damage is actually an important part of the adaptation process because the subsequent repair makes the muscles stronger and more resistant to future damage. Nevertheless, athletes and sports clubs are always seeking ways to minimise the damage or to speed the recovery process.

One strategy that has become very common in elite and sub-elite sports is cold water immersion (CWI). After intense exercise, athletes use CWI in temperatures between 10–15 °C for up to 15 min in the belief that muscle damage will be restricted and recovery enhanced so that subsequent athletic performance will be optimised.

However, there is little scientific evidence to support this practice and we therefore have set out to investigate the physiological response to CWI and establish whether cold water immersion really can reduce muscle damage and soreness to improve subsequent exercise performance.

How it works?

We will randomly assigned you to one of the group, which is cold water and exercise, OR exercise group

What is involved? - Cold Water and Exercise Group

Following a 12-hour overnight fast, we will take your height and weight. Then we will collect a blood sample. We will measure your leg strength (just 1 leg) using a mixture of voluntary and electrically stimulated contractions on a specialised strength-testing machine used by

Continue Appendix 6

sports clubs and physiotherapists. After this, you will perform 30 min of exercise stepping up and down on a step that is 40 cm high wearing a weight jacket (20 Kg). We will then ask you to sit in a bath for 15 minutes that has cold water (between 11-13 °C) filled up to your waist. We will record your heart rate and blood pressure while you sit in the bath. Finally, we will collect blood sample again and measure the muscle strength.

We will ask you to complete a questionnaire about how you feel and test your pain level. We will ask you to return to the labs 24 h, 48 h after the exercise to provide a blood sample, test your strength and complete a quick assessment to tell us whether you are experiencing any muscle soreness.

What is involved? - Exercise Group

This will be identical to group one, except no cold water will apply to the participant.

Are there any risks in taking part in the study?

The people who take part in this study will be familiar with exercise and probably also familiar to the sensations of aching legs the day after exercise. You might feel a little fatigued and possibly weaker, but this should not interfere with your usual daily activities.

Some people find the electrical stimulation of the muscle to be unpleasant due to the muscles being contracted quickly without you having to activate your muscles voluntarily. However, this is a very quick and easy test involving less than 10 contractions and after some familiarisation at very low intensity, most people tolerate the stimulation without any problem. If you do not like the stimulation then we will not continue with it.

In taking part in this study, it is likely that one or both of your legs might ache for one or two days afterwards. The reason is that whenever people do any heavy exercise, such as performing maximal strength contractions, the muscles become slightly damaged. The muscles then undergo a repair process and are subsequently stronger (this is the training-effect). This sensation of aching legs is felt most when you are sitting around and not very active, but when you begin to walk the legs will no longer ache. The sensation will last for around 2 days.

During all exercises and tests, and at the 24 and 48-hour follow-up, you will be monitored and questioned for signs of discomfort, injury or adverse reactions by a qualified physiotherapist.

If you feel any symptoms not described in this information, or if you feel you have been treated unfairly, please contact Noorah Alshoweir by email: nalshoweir@gmail.com or phone: 07787014341

Continue Appendix 6

CWIK Study

The use of cold water immersion to enhance post-exercise recovery in sports

Appointment times available:

	Day 1	Day 2	Day 3
1	9.30 – 12.30	9.30 – 10.15	9.30 – 10.15
2	10 – 1	10.15 – 11	10.15 – 11
3	10.30 –1.30	11 – 11.45	11 – 11.45
4	11 – 2	12 – 12.45	12 – 12.45
5	11.30 – 2.30	12.45 – 1.30	12.45 – 1.30
6	12 – 3	1.30 – 2.15	1.30 – 2.15
7	12.30 – 3.30	2.15 – 3	2.15 – 3
8	1 – 4	3 – 3.45	3 – 3.45
9	1.30 – 4.30	3.45 –4.30	3.45 –4.30

	Name (Capitals)	Date of Birth (*ddmmyy*)	Preferred appointment time [1 - 9] Day 1	Day 2	Day 3
1					
2					
3					
4					
5					
6					
7					
8					
9					

Appendix 7: Participants Record Form

Participant Group-

Participant ID-

CWIK Study

Participants Test Sheet CYBEX (Trail 2+3)

Height (CM) ………….
Weight (Kg) ………….

Cybex

MVC 90
Pre_ ……… …………….. ………………
Post 30 min …….. ………… …………..
Post (24 h) ……….. ……….. ………….
Post (48 h) ………. ………… …………..

FVC

Force	Pre_ CWI	(30 min) Post CWI	(24 h) Post CWI	(48 h)_ Post CWI
60				
120				
180				
240				
300				
360				
420				

Appendix 8: Participants Feedback Form

Participant Group-
Participant ID–

CWIK Study

Participants' Feedback

Personal Details

Subject ID: ………………

Sex: a) Male b) Female

D.O.B : ………………

Type of physical activities:

Basketball ____ Bowling _____ Boxing _____

Cycling ____ Running _____ Swimming ____

Tennis ____ Football _____ Gymnastic ____

Health / Fitness workout ____ Weight training / body building ____

Others: _________________

How often do you exercise per week?

1 2 3 4 5 6 7

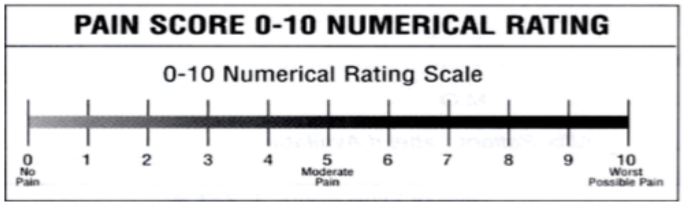
How many hours per week do you devote to exercise?

1—3 4—6 7—9 10– More ____

Based on the treatment that you have received today. Please answer the following questions?

1. How intense is your pain post exercise?

Continued Appendix 8



2. Have you use cold water immersion after exercise before? Yes No

If yes, how?
If not, why not?

....
....
....

1- Do you feel the cold water immersion duration is:

Enough Too long Too short

2- Was the temperature:

Cold enough Too cold Not cold enough

3- Did you find the position comfortable?
Yes No

If not, what position would you prefer?

....

4- What did you feel physically and emotionally during the experience?

....
....
....

5- What was your expectation of the experience?

....
....

Continued Appendix 8

6- Did you feel unsafe during the experience? Yes No

…………………………………………………………………………………………
…………………………………………………………………………………

7- If you stopped the intervention early – why did you do this?
…………………………………………………………………………………………
…………………………………………………………………………………

8- Would you use it as a treatment? Yes No
Why?
…………………………………………………………………………………………
…………………………………………………………………………………

Thank you for filling out the questionnaire

Appendix 9: Participants Perception Form

Participant Group-
Participant ID-

CWIK Study

Participants' Perception of the effects of CWI

Questions asked to the patient without them knowing you are recording the answer **at the**

- **30 mins test***
- **24 hours test***

*circle the appropriate test

After baseline strength testing (Static+dynamic) and **before** the CWI:

1. Dynamic test

Do you believe you will do better in the strength tests following the CWI? Yes / No
[circle answers]

Why?

…………………………………………………………………………………………………
……………………………………………………

Additional comments:

…………………………………………………………………………………………………
…………………………

2. Static test

Do you believe you will do better in the strength tests following the CWI? Yes / No

Why?

…………………………………………………………………………………………………
……………………………………………………

Additional comments:

…………………………………………………………………………………………………
…………………………

Continued Appendix 9

After CWI and **during strength testing** (Static+dynamic):

3. Dynamic test

a. Compared to before the CWI, how do you feel you are performing in the strength test?

Better / no different / worse

Why?

……………………………………………………………………………………………………
……………………………………………………

b. During the strength test, how does your thigh feel?

Stronger / no different / weaker

Additional comments:

……………………………………………………………………………………………………
…………………………

c. During the strength test, how does your thigh feel?

More comfortable / no different / less comfortable

In what way/can you explain how:

……………………………………………………………………………………………………
…………………………

4. Static test

a. Compared to before the CWI, how do you feel you are performing in the strength test?

Better / no different / worse

Why?

……………………………………………………………………………………………………
……………………………………………………

b. During the strength test, how does your thigh feel?

Stronger / no different / weaker

Continued Appendix 9

Why?
……………………………………………………………………………………………
………………………………………………….

Additional comments:
……………………………………………………………………………………………
………………………….

c. During the strength test, how does your thigh feel?

More comfortable / no different / less comfortable

In what way/can you explain how:

……………………………………………………………………………………………
………………………….

Appendix 10: Participants General Experience Form

CWIK Study - Day 3 Questionnaire

To all participants - Digitally recorded (preferable) or written answers
Pt ID________________

Cold Water Participants only
1. How did you feel when you were told you were going in the ice bath?
____
2. After the 20 min of step-ups, did your feelings about going in the ice bath change? – if so, why?
____
3. How do you feel today, generally?
____
4. How does your exercised leg feel today?
____
5. Do you think it would have felt different if you ***had not*** gone in the ice bath? – if so, why/in what way?
____

Exercise only participants only
1. How did you feel when you were told you were ***not*** going in the ice bath?
____
2. After the 20 min of step-ups, did your feelings about ***not*** going in the ice bath change? – if so, why?
____
3. How do you feel today, generally?
____
4. How does your exercised leg feel today?
____
5. Do you think your leg would have felt different if you ***had*** gone in the ice bath? – if so, why/in what way?
____

Appendix 11: Blood Pressure and Heart Rate Measurement Form

Participant Group-
Participant ID–

Cold Water Immersion Knowledge

CWIK Study

Weight (Kg) _________

Height (CM) _________

Heart Rate

Base line __________

I _min _________ 5_min __________ 10_min _________

15_min __________

BP

Base line __________

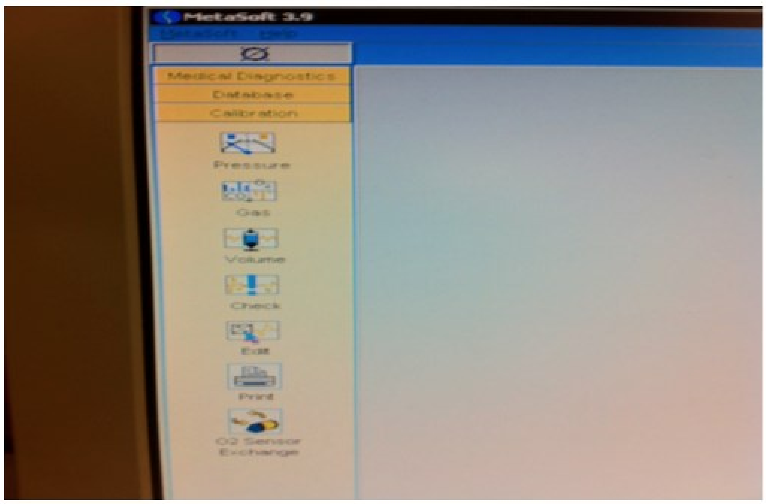
I _min _________ 5_min __________ 10_min _________

15_min __________

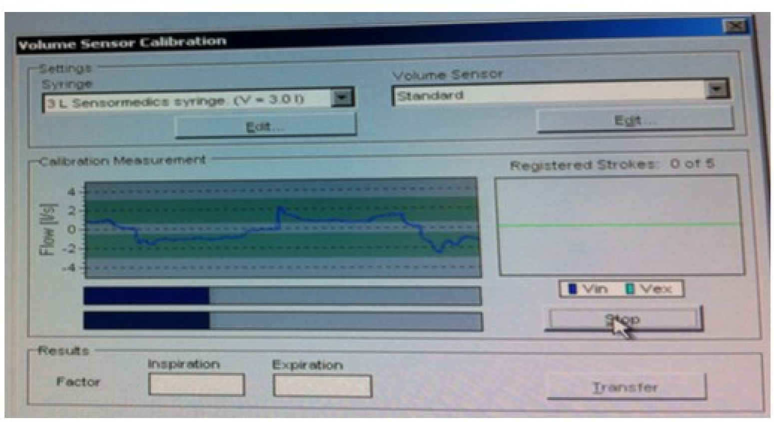
Appendix 12: Cortex Metalyzer Collaboration

Cortex Metalyzer (VO2 max Protocol)

1. Switch on PC and cortex system
2. Double click metaoft 3 icon on the screen
3. Enter username and password , both ‘Cortex’ (case sensitive)
4. On the left hand navigation bar, select calibration. This screen will appear



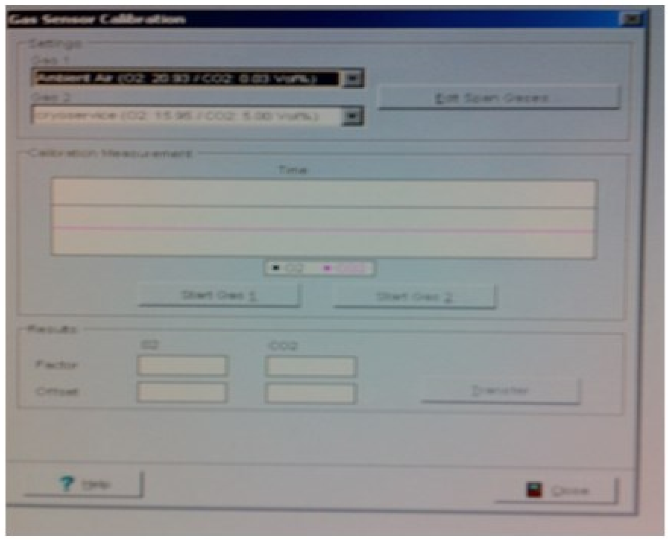
5. Assemble breathing valve and mouthpiece with gloves on. Connect the mouthpiece with the syringe. Select Volume in the calibration menu, and press start. A blue line starts to move left to right. Open and close the syringe in time with this line as it shown to the picture



6. Successful strokes will be record provided the stroke rate, once it appears in result section, press transfer.

Continued Appendix 12

7. Next, calibrate gas sensor. You will see the cortex measure gas every 10 sec. Click start gas 1, this is will be with ambient air and wait until is done.



8. Connect the cortex device line to the tedlar bag, click start gas 2, open the bag by unscrewing the metal tap a few turns, and wait until it completes.

9. Click transfer then close. Calibration is now complete

10. Click medical diagnosis and select new test subject and fill the required information.

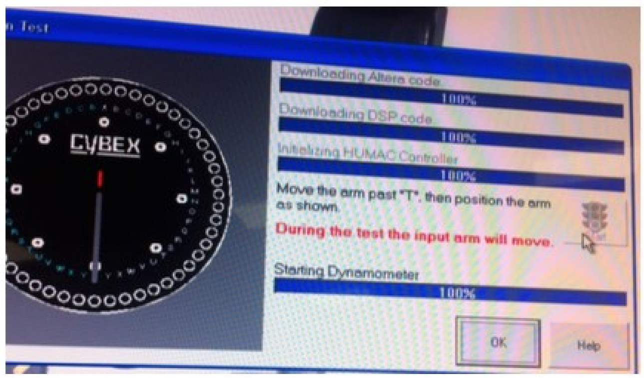
Appendix 13: Cybex Adjustment Procedure

Standard Operating Procedure Cybex dynamometer

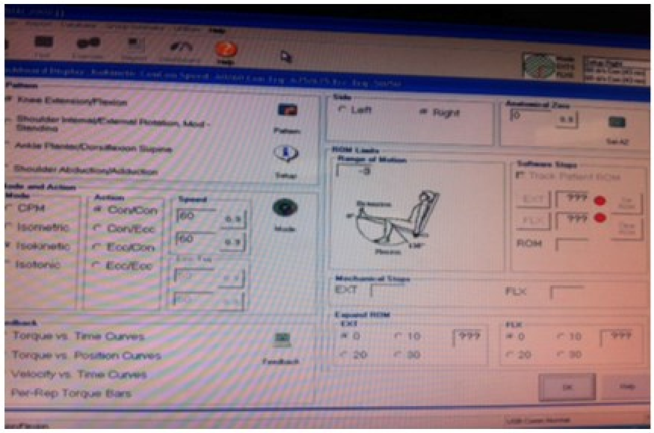
A Cybex isokinetic dynamometer will be used to evaluate the concentric strength of the knee extensors and flexors. Isokinetic dynamometers test muscle performance as the participant applies force against a continuously moving mechanical arm. This is in contrast to isometric tests, which require application of force against an immovable mechanical arm or lever.

Procedure:

- Switch on the Cybex machine
- Switch on the computer screen for the cypex and the Mac computer for the analysis
- Click HUMAC2008 from the screen
- It will appear this picture



- Position the arm as shown in the picture the red line
- Click start and wait until starting dynamometer load to 100%
- Click ok, then cancel to other window
- Click Dashboard to start the test. You will then be at the main screen, which will look like this:



- Position the participant on the cybex
  - Check that the seat angel is set to 85°
  - Ask participant to sit up straight with hip square and back of knees touching front of seat
  - Adjust seat back to participant by using the adjusting handle at the back of the seat
  - Adjust chair position so that the participant knee axis corresponds to the dynamometer axis. The axis of dynamometer should line up with the knee lateral epicondyle. You can adjust it by loosing the handle underneath the chair. Move the chair backward and forward then lock it.
  - Tight the ankle strap and shoulder belt.

- Set anatomical zero with participant leg to full extension then press set button
- Click track patient ROM
- Set ROM by asking the participant to extend the leg to the maximum range then press set ROM then maximum flexion then press again set ROM.
- It will appear in the mechanical stop box a letter for ext and flex.
- Adjust the wheel to the same letter that appears to the mechanical stop box.

- Select from mode and action the test that you require and adjust the speed.
- Click start to begin the test.

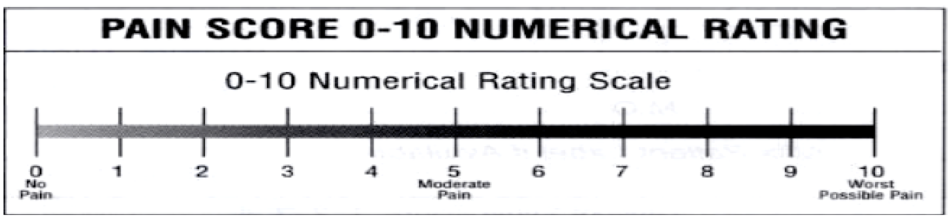
Appendix 14: VAS measurement

Participant ID-

CWIK Study

Pain Score

How intense is your pain post exercise?



PPT Vastus lateralis

	Right			Left		
Point	1	2	3	1	2	3
Force						

Comments

____

____

____

____

Appendix 15: Focus Group Questions Draft.

Questions

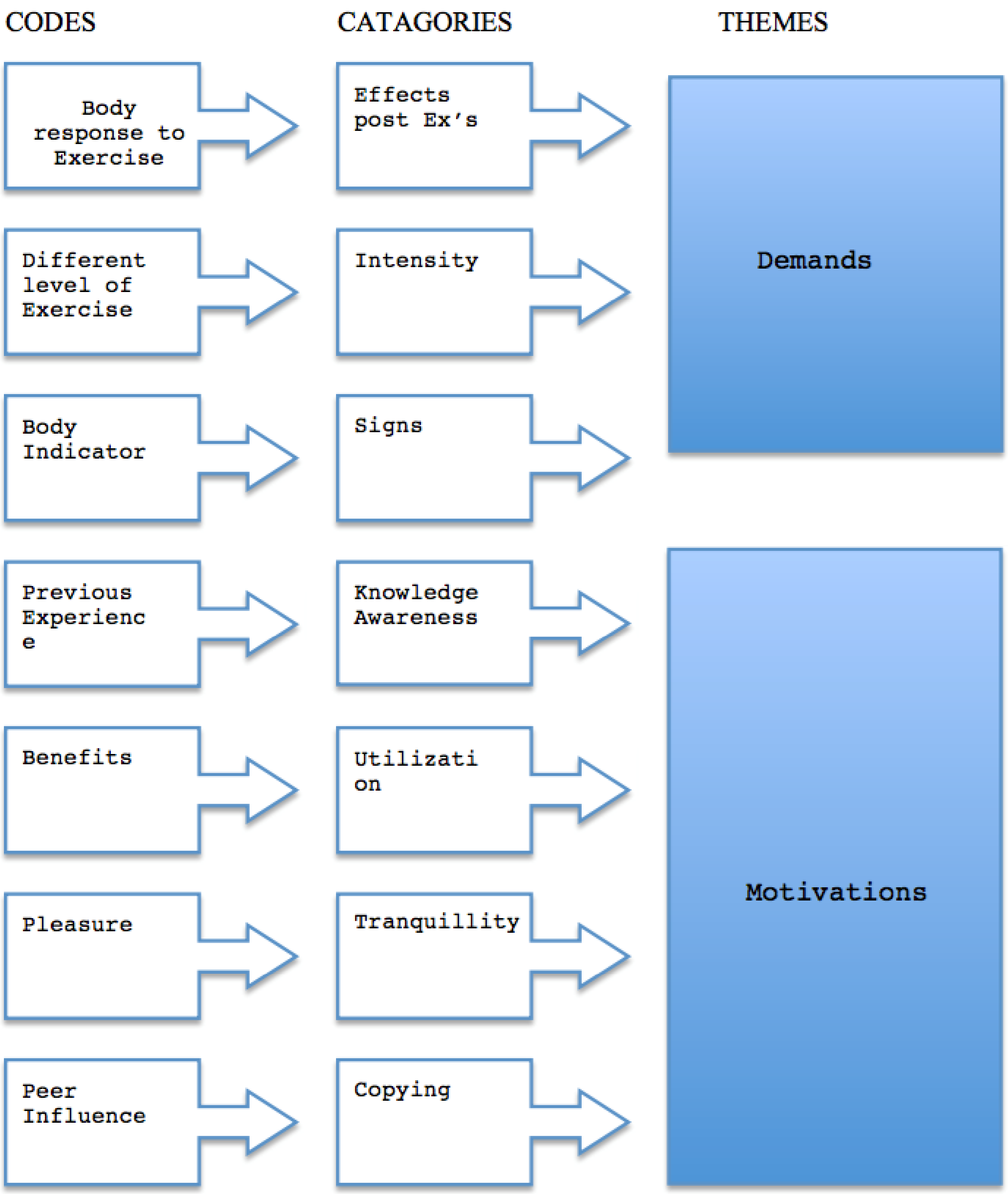
1. What do you think of cold water as a treatment for soreness after exercise?
2. Do you believe that your pain would be less after CWI?
3. Do you think that a longer period of cold water would be better?
4. Would you say that you are satisfied with the results? Why?
5. What did you generally feel after you finished the treatment?
6. How can you rate your pain after CWI? Explain why you give this rate?
7. Is there anything you would like to add about your CWI experience?

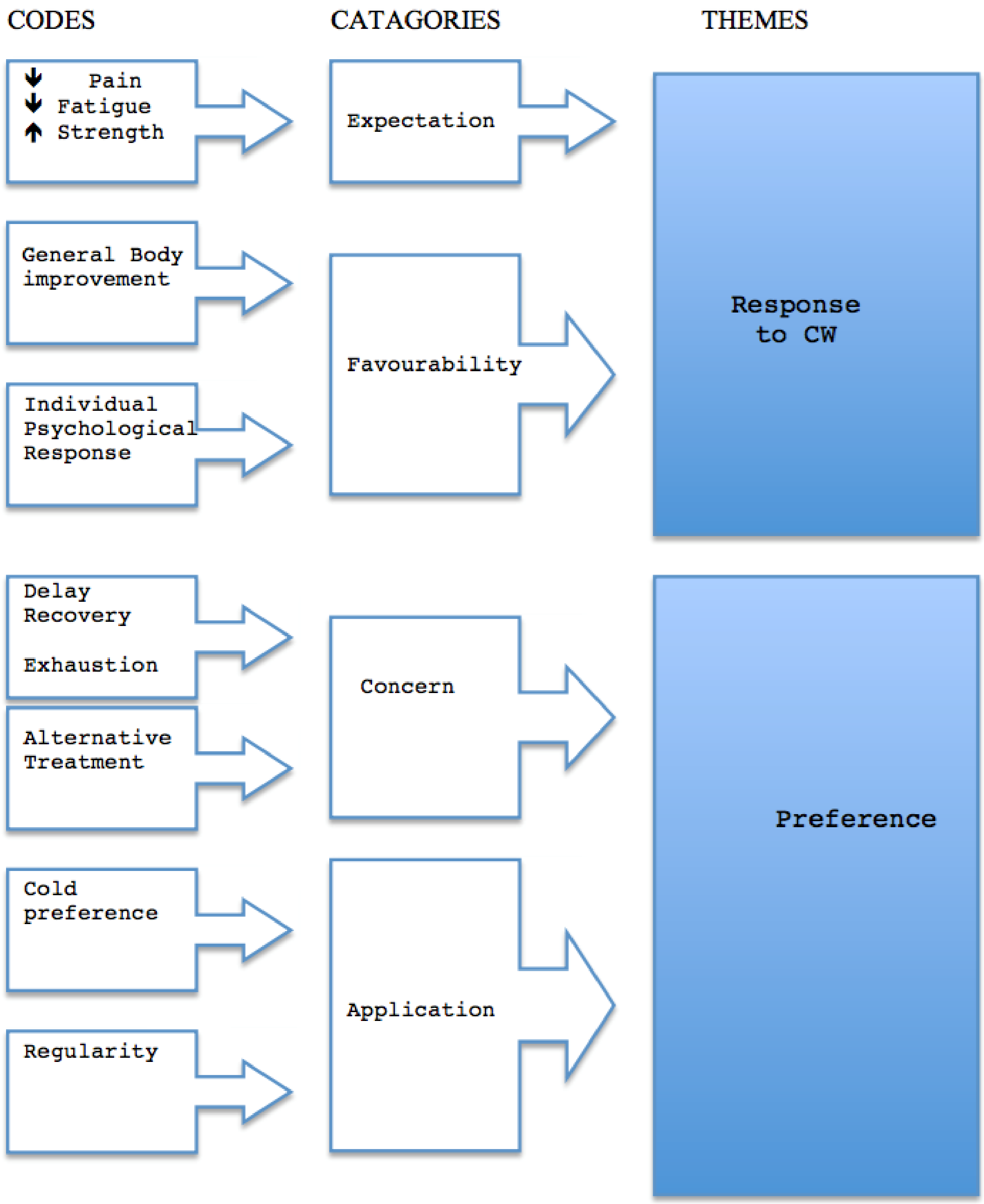
Appendix 16: Focus group questions

- Welcome / Introduction.
- Consent.
- Introduction to and consent to use the audio recorder.
- Why have you decided to participate? Did you have any personal reasons?
- Have you got a gut instinct before we provide you with your own data feedback?
- Is the CWI better in the whole body sense or is it merely your leg that feels better?
- What forms of exercise have you done in pre-season?
- Do you want to tell me how you felt when you got your group allocation?
- Does CWI effectively impact upon your muscle function?
- How effective do you feel ice is for overall physical improvement?
- Do you feel that you could return to training faster following CWI than without?
- Which application of CWI do you think is better: extremely cold for a short period, or merely cold for a long period?
- How often would you have the ice bath? Would you have it every week or would you have it every training session?
- Is there anything you would like to add about your CWI experience?
- Summary and close

Appendix 17: Focus Group Themes

Themes identified from participants perception of CWI




CODES
CATAGORIES
THEMES
↓ Pain
↓ Fatigue
↑ Strength
Expectation
General Body improvement
Individual Psychological Response
Favourability
Response to CW
Delay Recovery
Exhaustion
Alternative Treatment
Concern
Cold preference
Regularity
Application
Preference