PERCEPTIONS AND CURRENT PRACTICE *VS* EVIDENCE-BASED EFFECTS OF WHOLE-BODY CRYOTHERAPY ON FATIGUE AND RECOVERY IN ELITE RUGBY PLAYERS.

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ii. Abstract

The primary aim of this thesis was to advance the current field of knowledge regarding whole-body cryotherapy (WBC) and its influence on fatigue and recovery in elite rugby players. The secondary aim of this thesis was to explore the perceptions and current use of WBC in an elite contact sport population.

A systematic review analysed fifteen studies specific to the use of WBC following fatiguing physical activity. Despite the body of evidence reflecting low-to-moderate quality, WBC appears to result in perceived and symptomatic benefits, with some indications of positive change to recovery *via* physiological and inflammatory measures. Endocrinological responses were shown to be vastly under-researched and required clarification.

A qualitative survey formed the basis of exploring the beliefs and perceptions of fatigue, recovery and current practice of WBC within elite contact sport environments. The key findings highlighted that (i) recovery from evening match or training activity was frequently impacted due to reduced perceived sleep quality; (ii) the perceived benefits of WBC enhanced sleep quality for those who had experienced WBC; (iii) the beliefs of those who had not experienced WBC was that they would highly likely adopt its use on the basis that sleep quality and psychological wellbeing would be enhanced in order to benefit recovery; (iv) the adoption of WBC as a regular practice-based modality for recovery is low mainly due to cost, logistics and lack of evidence-based information supporting parameters of implementation.

Two experimental, field-based studies were conducted using an elite Rugby League team from the European Super League. The first was an observational study and had the purpose of analysing the endocrinological (cortisol and testosterone) responses to a nine-day period of competition involving match and training activity and regular WBC exposures. These have been shown to be reliable indicators of catabolic and anabolic basal control in response to high intensity physical activity. The use of WBC post-training appeared to attenuate anabolic status, whereas following matches, anabolic signalling was enhanced following WBC. The second study was a participant-

controlled design comparing post-match recovery inclusive of WBC to regular recovery processes without WBC. Two, three-minute exposures to WBC showed significant alteration to the endocrine profile of players at 60 h post-match in that greater concentrations of testosterone were found without concurrent change in cortisol. A significant effect was shown after one exposure to WBC, albeit to a lesser degree. Without WBC, the recovery of endocrine and biochemical profile was not reached by the end of the 60-h sample period.

In summary, this thesis supports the use of WBC in that it appears to hold benefits for athletes in their recovery from elite level collision sport. Greater benefits appear more supported following matches, with a lesser extent, post-training sessions. Medical practitioners, coaches and sport scientists/conditioners should look to optimise the influence of WBC though applying two, 3-minute exposures as close to the cessation of high-fatiguing activity as possible. WBC has the potential to improve the wellbeing and recovery of collision sport athletes, however, the underlying mechanisms involved remain undetermined despite the observation of a desirable change in endocrinological profile.

Further study should look to specifically investigate the effects of WBC relative to the time of day of activity given that indications in this study from a perceived perspective suggest that effects may be relative to activity and time of day. This area currently holds negligible supporting evidence and will assist to inform the implementation strategy of WBC in the team environment.

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Chapter 1

Introduction

1.1. Background

Rugby is a popular high intensity team sport played under two codes, rugby union (RU) and rugby league (RL). Recent figures from World Rugby and the International Rugby League (IRL) suggest that there are 9.6 million participants worldwide at various levels of competition in 128 countries in RU, with RL represented across 68 countries and 55 member nations recognized by the IRL (IRL, 2020; World Rugby, 2019). The 2019 Rugby Union World Cup in Japan was the most watched rugby event ever with an audience of more than 857 million people worldwide (World Rugby, 2020a). The 2021 Rugby League World Cup pre-COVID-19 was expected to draw its largest audience and participation figures with the concurrent running of men's, women's and wheelchair competitions. The 2019 World Rugby financial statement reported a global total revenue of £381.5 million (World Rugby, 2020b). From a domestic perspective the Rugby Football League (RFL) reported an overall turnover of £22.9 million in 2019 (RFL, 2019).

Both rugby codes are played at junior, amateur, semi-professional and professional levels of participation, over regional, national and international competitions (King et al., 2009). As the nature of the sports involve intermittent, high intensity bouts of physical activity, the sports also require participants to compete with a combination of muscular strength, endurance, speed, acceleration, agility, flexibility and aerobic endurance (Gabbett, 2013). The duration of a senior match is 80-min played across two, forty-minute halves. Depending upon position (i.e., a general classification of forwards and backs), players will cover a total distance of 4-8 km during this time with the majority of this distance at low intensity levels. Time-motion analyses in RL show that players spend between 12 and 17 % of relative time on the field performing high intensity work such as sprinting and tackling (King et al., 2009). In rugby union, a similar figure has been observed with forwards performing 13 % of match time at high intensities. Backs are shown to have a lower work-to-rest ratio (in excess of 1:20), whereas forwards are significantly higher at 1:6 in RL and 1:7 in RU (King et al., 2009; Deutsch et al., 2007).

1.2 The 'fatigue – recovery' issue in rugby

A key aspect of rugby involves tackling and collisions, and therefore in conjunction with the high intensity running requirements it is inevitable that structural damage, injury and fatigue are likely following rugby-specific activities (RSA). Injury incidence covering time-loss injuries has most recently been reported as 85 per 1000 h in RU, and 78 per 1000 h in RL, thereby supporting the fact that the injury risk in RSA is high in comparison to other sports (Fitzpatrick et al., 2018; EPRISP, 2019). Clear evidence of post-match fatigue has been published, where restoration to a pre-match state can take between 48 h and five days following a competitive match (McLean et al., 2010; McLellan et al., 2010; Twist & Sykes, 2011).

Rugby players frequently train two or more times daily, for two or more consecutive days, which creates a high-density weekly schedule (McLean et al., 2010). This impacts on recovery time and therefore players are reported to remain in a state of fatigue throughout an in-season training week (Quarrie et al., 2017). Following matches, players need to recover quickly in order to recommence training and prepare for the next scheduled game (Johnston et al., 2013). Competing whilst fatigued can heighten the risk of injury, and if associated training loads are cumulatively high, so too is the concurrent risk of overtraining (Gabbett & Jenkins, 2011; Quarrie et al., 2017).

As a result, the impact of rugby injury is potentially severe for the participant, their club, and public and private health systems. Significant long-term career limitations, medical costs and loss of income have been associated with major match injuries across all levels of the sports. King et al. (2019) reported financial entitlement claims for RU and RL players in New Zealand to reach up to £5500 per moderate-to-serious injury. In the UK, the most recent figures for average time loss severity per injury in the elite games is 37 days for RU, and 34 days for RL (Fitzpatrick et al., 2018; EPRISP, 2019). Therefore, this average injury time loss (and considering the high incidence rate) can drastically impact upon team selection and create a heavy financial burden for teams where wages are essentially covering injured players unavailable for competition.

Given that the underlying risk factors outlined above are well established in the rugby literature, the importance of recovery from RSA is therefore paramount to all stakeholders in these sports given the physical and mental wellbeing perspectives through to financial implications. Therefore, the extent of adaptation to RSA is dependent upon the quality and time frame of an athlete's recovery, which brings forward the focus of this thesis. As such, the need to optimize recovery and adaptation is vital to the large populations involved in the game, and over the past 15 years, a number of studies have observed the impact of varied recovery modalities specifically in response to RSA (Tavares et al., 2017). The review by Tavares and colleagues (2017) describes a range of recovery modalities utilised post-RSA, such as active recovery, cold-water immersion, contrast water immersion, compression garments, and, to a lesser extent, whole-body cryotherapy (WBC). The success of improving recovery with these different modalities is variable, as is the broad range of measurements used to analyse the extent of recovery.

Ultimately the main goal in the competitive arena is performance driven i.e., to win the match. Team success is highly facilitated by maintaining players' match-fit status for the subsequent fixture, and all other matches of the season. There is evidence that optimized recovery and preparation for competitive events or sports fixtures brings a high probability for positive performance outcomes (McLean et al., 2010).

In practice, athletes and sports teams tend to readily implement products and equipment with the intention that any tool that enhances the athlete's perception of an enhanced recovery and subsequent performance preparation even by the smallest of margins (i.e. the 'marginal gains' concept, Patel, 2016), is beneficial. However, the underlying issue is the lack of quality studies that inform the use of these modalities, and therefore, despite the study by Tavares et al. (2017) no strong body of research is able to clearly evidence the true impact (if any) that a particular modality has.

1.3 Whole-body cryotherapy as a recovery tool

WBC is a popular modality to be recently employed in elite, professional sport (Haq et al., 2018). WBC is a short duration (2-3 min) exposure to extreme cold air of -110 to -180 °C in temperature-controlled units, either through electric cooling systems or by means of liquid nitrogen (Banfi et al., 2010; Laza, 2019). The background of WBC research from the 1980s and 1990s largely involved osteo- and rheum- arthritic patients, showing temporarily improved symptoms of muscle pain, joint stiffness, sleep quality and general feelings of wellbeing (Yamauchi et al., 1981; Taletavičienė et al., 2012). The recent use of WBC in contact sports teams and by individual athletes has attracted attention from the mainstream media across the UK, USA, and Australia (BBC, 2012; Fox Sports, 2015; The Guardian, 2017). As a result of this, private healthcare providers of WBC have become well established, and promote the use of WBC with athletes in their practices (Cryoaction, 2019; BMI Healthcare, 2020). Bouzigon et al. (2016) reported that 15 manufacturers of extreme cooling devices existed across the world, a number which has undoubtedly increased since this study was published. The application of WBC has been reported to consist of a single treatment exposure, two exposures consecutively, or even daily, in its attempts to positively impact recovery status following intense physical activities (Costello et al., 2012; Ziemann et al., 2014; Wilson et al., 2018). This variety in application methods of WBC requires clarity for its optimal use with respect to timing, frequency and exposure dosage. Especially since no controlled, dose-response studies have yet to be conducted. Only scant evidence analysing WBC session frequency (Lubowska et al., 2010) or exposure time (Selfe et al., 2014; Fonda et al., 2014) exists. Since then, various protocols of application have been employed, however, without sufficient mechanistic evidence to underpin their inclusion in studies. Furthermore, a broad spectrum of measurement has been used to quantify the extent of recovery. Therefore, in context of post-activity recovery, a pooled analysis of common measures used across studies is required to establish where measures of biological restoration, for example biochemical and endocrine markers, are under-investigated.

The manufacturing of WBC technology has also increased, such that various forms of delivering extreme cold air therapy is available to the public as well as athletes. These

forms of delivery are typically termed whole- and partial body cryotherapy (PBC). The main difference being evident when the chamber is either a fully enclosed unit, or open to the external environment. In the former, the user's full body and head is subject to the extreme temperatures (WBC), whereas in the latter, only the user's body is subject to the cold air whilst the head is not (PBC). An immediate issue arises when providers of extreme cold environment refer to both modalities as WBC, when the comparable effects of both WBC, and PBC have not been directly investigated.

1.4 Current perceptions and practice of WBC

Whilst it appears that the anecdotal use of WBC in athletes is high (Costello and Leeder, 2013; Partridge et al., 2019), the research underpinning the effects of WBC is still growing. For athletes, coaches and practitioners alike, the reasoning for implementing WBC may be driven by different factors (from scientific, to anecdotal or perceived benefits). Generally, athletes and coaches strive for performance advantages, whilst practitioners look to inform and optimize their practice strategies (Haq et al., 2018). To date, however, there is no strong evidence which clarifies the beliefs, perceptions and practices of WBC from the perspective of the athlete, coach, and practitioner. This therefore warrants investigation, particularly in elite sport settings where each of the three aforementioned stakeholders involved in optimizing team performance can benefit from an informed approach. There is very limited practice-related information available relating to when athletes use WBC during their schedules, and so naturally, this requires clarification in order to identify the potential of a timing (time of day, and/or relevant time to RSA) influence in its use. The biological responses to the use of WBC for recovery specifically in an elite team sport setting is also unclear, nor has the dose prescription (i.e., frequency of exposure) of WBC been compared (Haq et al., 2018). Understandably, due to the nature of the professional sport environment, internal validity and study design are difficult factors to regulate since the daily schedule of team training and competition is dictated by team management (Coutts, 2017).

Therefore, the overall goals of this thesis were to investigate the perceptions and current practice *vs* the evidence-based effects of WBC upon fatigue and recovery in elite rugby players.

1.5 Aims and Objectives

To surmise, the **aims of this thesis** are as follows:

1. To systematically analyse the current body of research with respect to the effectiveness of WBC upon recovery status following intense physical activities.

2. To investigate the current perceptions, beliefs and practices of WBC within elite collision sport team settings in order to analyse its current use.

3. To investigate the effects of WBC upon fatigue characteristics and recovery status following intense physical activities during a period of Rugby League training and competition.

4. To investigate the dose response of WBC and the effects upon recovery following competitive Rugby League matches.

5. To inform the application of WBC in elite rugby environments with consideration to treatment timing and dosage following RSA using an evidence-based approach.

The **key objectives** that this thesis will employ in meeting these aims are as follows:

1. Review the evidence underpinning WBC modalities and protocols in relation to fatigue and recovery following strenuous physical activity using a systematic review and pooled data analysis.

2. Employ a qualitative, survey-based study design to establish the current perceptions, beliefs and practices of WBC from the perspective of the athlete, coach, and practitioner in elite, collision sport teams.

3. Employ a field-based, quantitative observational protocol to establish the effects of WBC in an elite rugby team setting considering the competitive and training environments.

4. Employ a field-based, quantitative controlled experimental trial to establish the dose response of WBC immediately following rugby league competitive fixtures.

5. Synthesize the findings of the qualitative and quantitative studies in order to inform the prescriptive use of WBC in an elite rugby environment with sound scientific underpinning.

Chapter 2

Literature Review

2.1 Structure of the literature review

The intention of the review is to familiarise the reader with the physiological concepts and evidence on which this thesis is based.

The review begins with an exploration of the concept of fatigue and its classical mechanisms of manifestation. A discussion of determinants, processes and characteristics of fatigue is provided so that a broader review of published literature can be explored across its complex presentation.

Given that recovery from fatiguing physical activity is typically judged by the completeness and time of return to pre-fatigue status, the signs and symptoms of fatigue and markers of recovery are reviewed accordingly. This covers neuromuscular measurements, as well as hormonal and biochemical markers to represent various human responses to fatiguing stimuli. Accordingly, the reliability of these markers is discussed in order to justify their inclusion within the various studies presented later in this thesis.

The review concludes with coverage of the current base of literature evidencing the effects of whole-body cryotherapy. This section explores its influence upon thermal and regulatory responses of the body and updates the reader on its use in varying physical activity contexts. The effectiveness of whole-body cryotherapy in field- and laboratory-based studies is discussed whilst highlighting the areas of research which require more focus. The extent of real-world practice using recovery modalities such as whole-body cryotherapy for recovery is briefly reviewed and provides a basis on which to justify the collection of practice-based information in this field.

2.2 Fatigue – definition and mechanisms

The term ‘fatigue’ is one that is broadly used to describe the manner in which the performance of a task in a wakeful state deteriorates, becomes suboptimal, or more challenging to maintain, as a result of the events preceding or during that task (Allen et al., 2008; Taylor & Gandevia, 2008). It could be argued that in light of the physiological and psychological complexity of the concept, fatigue merely exists as conscious interpretation of homeostatic changes in the body (Noakes et al., 2005), and ultimately manifests itself as a symptom (Balachander et al., 2014*)*. Noakes, in his comprehensive 2012 review paper, termed fatigue as a ‘brain-derived emotion that regulates the exercise behaviour to ensure protection of whole-body homeostasis’ (Noakes, 2012). The term itself can therefore be utilised and applied across a variety of contexts, and its meaning can refer to an alteration of optimal function in a number of physiological systems within the human body. These include the muscular, respiratory, neurological, endocrine, and immunological systems, and also in the context of the psychological state of the human body (Kennedy et al., 2013). In addition, a fatigue process can develop over broad temporal scales, for example across seconds, minutes, hours, weeks, or years, adding to its complexity, and therefore categorised as temporary, prolonged (more than a month up to six-months), or chronic (over six-months, Balachander et al., 2014).

Classically, the classifications of fatigue affecting task performance has distinguished between ‘central’ or ‘peripheral’ processes, however, these mechanisms mutually interact with each other, and their individual focus should be avoided when considering the nature of a fatiguing task (Barry & Enoka, 2007). However, for the ease of describing these two overarching mechanisms, they are outlined below.

2.2.1 Central fatigue mechanisms

Neurological signals sent to muscles to perform tasks originate from the cerebral cortex and follow descending spinal cord pathways. Signals follow a co-ordinated distribution throughout the peripheral motor neurons, and therefore central fatigue encompasses the alteration of events that occur proximal to the neuromuscular junction (Amann & Calbet, 2008). The level of this central motor drive has been shown

to be subject to modulation in a number of ways as reviewed by Shei & Mickleborough (2013). Briefly, increased feedback activity from group III and IV afferents as a result of metabolic changes to muscle fibres precede motor cortex excitability. These supraspinal factors downregulate the resulting motor drive when this recognition of the change of metabolic status of muscle fibre occurs (Amann, 2011). Alterations in concentration of dopamine (decreases) and serotonin (increases) within the brain tissue have also been reported to contribute to a reduced motor drive. The resultant effect is that of reduced muscle force output, a disruption of motor unit co-ordination, the alteration of motor unit threshold recruitment, and the potential for muscle straining (Noakes, 2007; Stock et al., 2012; Contessa et al., 2016). At the extreme ends of the spectrum, tasks requiring maximal force output such as weightlifting, and endurance tasks such as ultra-distance running have been recognised as causative activities due to their impact upon neurological feedback and feedforward, and furthermore, upon resultant motor cortex excitability.

Muscle output requirements for maximal voluntary contractions (MVCs) such as heavy weightlifting tasks, demand that motor unit synchronisation is optimised in order to generate maximal muscle fibre recruitment. Any compromise in this process would therefore be recognised through a reduction of force output and electrical activity (Taylor & Gandevia, 2008). The task may not be completed, possibly with subsequent structural tearing to the myofascial system due to mechanical overload beyond the capabilities of the recruiting motor units and passive tissue properties of the muscle-tendon unit (Buckthorpe et al., 2014).

Long duration tasks such as ultra-distance running apply a cumulative load to the musculoskeletal system, where the effects upon neurological functioning are clearly evident (Degache et al., 2014). A Central Governor Model has been proposed to explain the fatigue phenomenon during such events whereby alterations in the periphery, whether structural, metabolic, or chemical provide feedback to the brain (the ‘Central Governor’). This can then downregulate resultant muscular activity in order to protect one’s homeostatic status and reduce the risk of injury or illness (Noakes, 2007). Recent discussions on this concept have included a psychological-physiological interaction which may also influence the Central Governor, and therefore an ‘Integrative Governor’ may be a more accurate theory of the entire fatigue

mechanism (St Clair Gibson et al., 2018). This concept is further alluded to in the discussion of psychological fatigue, below.

As such, it seems feasible that central fatigue mechanisms are secondary to perceptual stimuli or other, more localised stimuli at the working musculature which are detected and relayed as a result of peripheral mechanisms.

2.2.2 Peripheral fatigue mechanisms

It is deemed those factors affecting intracellular function of the muscle i.e., at or distal to the neuromuscular junction, rather than spinal or supraspinal factors are the contributors to the description of peripheral fatigue. A fundamental aspect to maintaining muscular output is the supply of adenosine triphosphate (ATP) to the working muscles, and furthermore the muscle cells maintaining their metabolic balance and infrastructure to accomplish excitation-contraction coupling, and sliding filament activation (Shei & Mickleborough, 2013). Detection of changes to these aspects are monitored by group III afferents and ultimately relay a muscle's current status to the brain. Furthermore, maintenance of muscular output is reliant upon effective processes at the neuromuscular junction of a muscle fibre, such as action potential transmission *via* acetycholine and calcium interaction in the motor end bulb (Keynes & Aidley, 2001).

Energy re-synthesis mechanisms (phosphorylcreatine mechanism, glycolysis, and oxidative phosphorylation) will all exhibit different fatigue phenomena, which limit the ATP production required to maintain muscular output. This is due to their predominance in ATP provision being dictated by exercise intensity (Westerblad et al., 2010). Accumulation of metabolites during repeated high intensity activity include inorganic phosphates (Pi) and hydrogen cations (H^+). These have direct effects upon ATP re-synthesis such as phosphofructokinase inhibition and interference with actin-myosin binding properties at the cross-bridge site (Debold, 2012). In particular, contraction velocity and muscle fibre power are reduced as a result of Pi and H^+.

Calcium (Ca^{2+}) sensitivity of muscle reduces due to a build-up of intracellular metabolites, therefore compromising the ability for the sarcoplasmic reticulum (SR) to

release Ca^{2+} for its binding to troponin. Reduced Ca^{2+} sensitivity results in lower forces being produced, particularly during the latter extents of the peripheral fatigue mechanism (Debold, 2012).

Relative hypoxia is an underlying factor within oxidative phosphorylation, since the electron transport chain requires oxygen as its final electron acceptor during its ATP re-synthesis mechanism. This occurs when arterial oxygen content reduces as a result of the cardiovascular system not being able to meet exercise demands. This is subsequently detected by group IV afferents (biochemical changes) in the muscle which potentially blunts the vasomotor areas of the brain (Amann & Calbet, 2007). A further possibility of reduced oxygen delivery is through gradual blood flow occlusion as a result of prolonged or repetitive muscle contractions (Barry & Enoka, 2007).

Glycogen is an essential substrate that maintains an adequate global energy supply to muscle contraction (Ortenblad et al., 2013). The impact of glycogen depletion upon fatigue appears to centre on the deterioration of SR release of Ca^{2+} despite the maintenance of ATP availability. Nielson et al. (2011) has furthered this by demonstrating that muscle glycogen depletion is more evident within subcellular localisation to intramyofibrillar stores rather than intermyofibrillar, and subsarcolemmal stores, although this was strictly applicable to trained individuals performing exhaustive exercise over a 1-h period. The authors postulated a role of sparing intermyofibrillar glycogen, of which type II muscle fibres maintain a greater proportion of its glycogen stores compared to type I fibres in an exhausted state. The translation of these findings into the notion of 'hitting the wall' as a performance identifier therefore relates to the ability to maintain the highest intensity as possible for as long as possible, and as such, is determined largely by an individual's aerobic capabilities and glycogen status.

2.2.3 Exercise-induced muscle damage

High intensity physical activity typically utilises eccentric muscle contractions to decelerate the body and absorb the forces created by body mass motion. Therefore, large forces are produced (higher than those of concentric or isometric type contractions) with a relatively lower metabolic cost (Semmler, 2014). The resultant

damaging processes of repeated eccentric contractions have been reviewed and evidenced clearly in the literature. McKune et al. (2012) summarises the structural alterations caused by such muscle actions into sarcomere disruption, disruption of muscle cytoskeletal elements, damage to the sarcolemma, impaired excitation-coupling mechanism, and loss of Ca^{2+} homeostasis. Decreased motor unit recruitment during eccentric contractions (due to a lower metabolic cost) result in increased force per fibre, thereby predisposing the contractile proteins to failure (Tee et al., 2007). Furthermore, 'weaker' sarcomeres within myofibrils become overstretched, and fail to reconnect their contractile elements, consequently affecting or extending to other sarcomeres (Ferreira-Junior et al., 2014). Where the extent of damage affects the myofibril as a whole, Ca^{2+} content within the SR surrounding the myofibril drops below normal level, then the amount released by each action potential is reduced. This consequently reduces the force response of the muscle fibre (Allen et al., 2008). To add to the muscle's detriment, if intrafusal muscle spindle fibres are also damaged, then the discharge of afferent feedback to spinal and supraspinal centres is altered. As such, the performer may need to utilise neural adjustments and altered motor patterning to maintain function. The level at which this occurs may depend upon the performer's fatigue level and ability to cope with the exercise stimulus (Nicol et al., 2003). Where task performance reduces, the stretch reflex and Hoffman reflex amplitudes also reduce, potentially as part of a protective mechanism, which utilises both peripheral and central fatigue pathways (Nicol et al., 2003).

2.2.4 Psychological fatigue

The processes and characteristics of fatigue due to physical activity are somewhat complex, varied and multifactorial, but are inherently interrelated with physiological fatigue. This is evident especially when tasks that are repetitive and prolonged with limited restoration affect behaviour, perception, motivation and sleep quality (St Clair Gibson et al., 2018). The forms of psychological fatigue that are outlined in the literature appear to be differentiated into mental and cognitive aspects, although sometimes confusingly referred to as the same thing. Ultimately, psychological fatigue is defined as a decline in ability and efficiency of mental or cognitive tasks that is caused by excessive mental, cognitive, or physical activities, or disease (Ishii et al., 2014). Ackerman & Kanfer (2009) offer an alternative definition for this type of fatigue

as the subjective state in which work declines after an extended time performing a cognitive task. Mental fatigue implies a change in psychobiological state, where the subjective perceptions of a task, or those preceding a task may coincide with, or manifest as a result of an alteration of physiological function (Akerstedt et al., 2004). Individuals with rather extreme conditions of prolonged fatigue (such as overtraining syndrome or chronic fatigue syndrome) display rather significant changes in psychological state and are beyond the scope and focus of this thesis.

There is sufficient evidence to support the notion that mental fatigue or excessive mental workload influences both localised muscular output, and athletic performance. Mehta and Agnew (2012) employed a 3 x 2 repeated measures design incorporating three levels of physical workload (intermittent bouts of low, moderate and high percentages of shoulder abduction MVC), and two levels of mental workload (absence and presence of an arithmetic task). Their findings highlighted significant influences of mental workload on muscular endurance measures, particularly at moderate (35 %) levels of MVC, and greater strength declines following all workloads (15, 35 and 55 % MVC). The authors also raised a key point which supports the recent Integrative Governor theory, in that while under normal circumstances (without mental influence), the onset of fatigue would typically be induced by peripheral mechanisms at lower levels of MVC. However, their findings suggest that since a greater strength decline also occurred following the 35 % MVC trial, then the arithmetic task was potentially pre-influencing central brain mechanisms which later determined altered central drive. Pires et al. (2018) provided recent support of this evidence in observing the reduction of cortical activation via encephalography (EEG) and performance decrements of a 20 km cycling time trial following a 30 min rapid visual information processing task. EEG activity during the time trial was significantly increased following the task compared to a control trial without the pre-cycling mental task, whilst the power output throughout was also reduced by 6.5 % (mean 16 W), and notably in the final 2 km by approximately 40 W. The authors also calculated an extremely large ($P = 0.03$) effect size because of the mental task.

Both studies, expectedly, measured perceptual characteristics of their respective participants. Mehta and Agnew (2012) employed the Swedish Observational Fatigue Inventory (SOFI), which assessed five fatigue dimensions: lack of energy, lack of

motivation, physical discomfort, physical exertion and sleepiness. Pires et al. (2018) utilised a shortened Profile of Mood States (POMS) using descriptors to measure anger, confusion, depression, fatigue, tension, and vigour following their mental task. The former study demonstrated significant effects upon motivation and sleepiness following the mental task, although interestingly, feelings of lack of energy, perceived exertion and physical discomfort were unaffected despite local muscular performance differences. The latter study found that, in particular, increased tension and a reduction in vigour contributed to an overall decreased mood state following the mental task applied prior to the cycling time-trial.

The evidence discussed here imply that the integrative mechanisms involve communication or influence of the prefrontal cortex upon pre/primary motor areas of the brain. This top-down modulation of neurological drive clearly involves high order cognitive control, and therefore the cerebral areas are subject to the effects of attentional tasks that require executive functions, decision making, sustained attention, goal-directed attention and response inhibition.

2.2.5 Alternative constructs of fatigue

The alternative suggestions to the classic physiological classifications of ‘central’ and ‘peripheral’ fatigue (and also psychological fatigue) are outlined by Barry & Enoka (2007). These are causative determinants which allow broader considerations to be applied to the descriptor, processes, and characteristics of fatigued state: (i) task dependency (ii) relation between muscle force and endurance time (iii) muscle wisdom (iv) perception of effort.

In brief, task dependency can encompass variations in contraction type, intermittent or sustained activities, gender and age-relative factors for a given task, extending to variations of intensity and time requirements of a particular task. Muscle wisdom refers to the alternative strategies that the neuromuscular system utilises based upon afferent feedback, variations in fatiguing tasks (task dependency) and subsequent tasks following the initial influential fatigue process. The perception of effort is rather more encompassing, whereby subjective interpretations of task requirements can be affected not only by the preceding three mechanisms, but also the daily state of the

individual, for example daily stressors, anxiety, sleep quality, immune function and perceived health status (Suda et al., 2009).

The multiple concepts and descriptors surrounding the complexity of fatigue creates some confusion around the actual construct of the term i.e., whether 'fatigue' itself is a process, a descriptor, a symptom/state, or a combination of all three. The definitions provided from the outset of this work imply that both process and state are implicit in the application of this phenomenon to real-world individuals and activities. The details above somewhat explain the determinants of fatigue (i.e., *process)* from a physiological perspective, and resultant psychological and performance-based manifestations (i.e. *characteristics*) of a fatigue condition. The restoration or recovery caused by the induced activity implies that the individual concerned is in a *state* of fatigue. The state of fatigue show signs and symptoms that indicate alterations in physiological, endocrine, histological, cardiovascular, neuromuscular, metabolic mechanisms, or mental state which are still returning towards their initial or pre-activity levels after the cessation of the fatiguing activity. Therefore, the definitions should perhaps consider the emphasis of the alignment between a fatigue process, resultant characteristics, and fluctuating signs and symptoms of what defines a greater or lesser extent of fatigue. The risk factors for the manifestation, and potential maintenance of a fatigued state, as suggested in Figure 2.1, should also be considered. Serious conditions such as overtraining, or chronic fatigue syndrome maintain a state of fatigue for prolonged time periods. In these conditions, a gradual adaptive change in one's physiology, endocrinology and immunology, and psychology leads to consistent reductions in activity performance (Purvis et al., 2010), or lessened ability to undertake or complete physical or mental tasks.

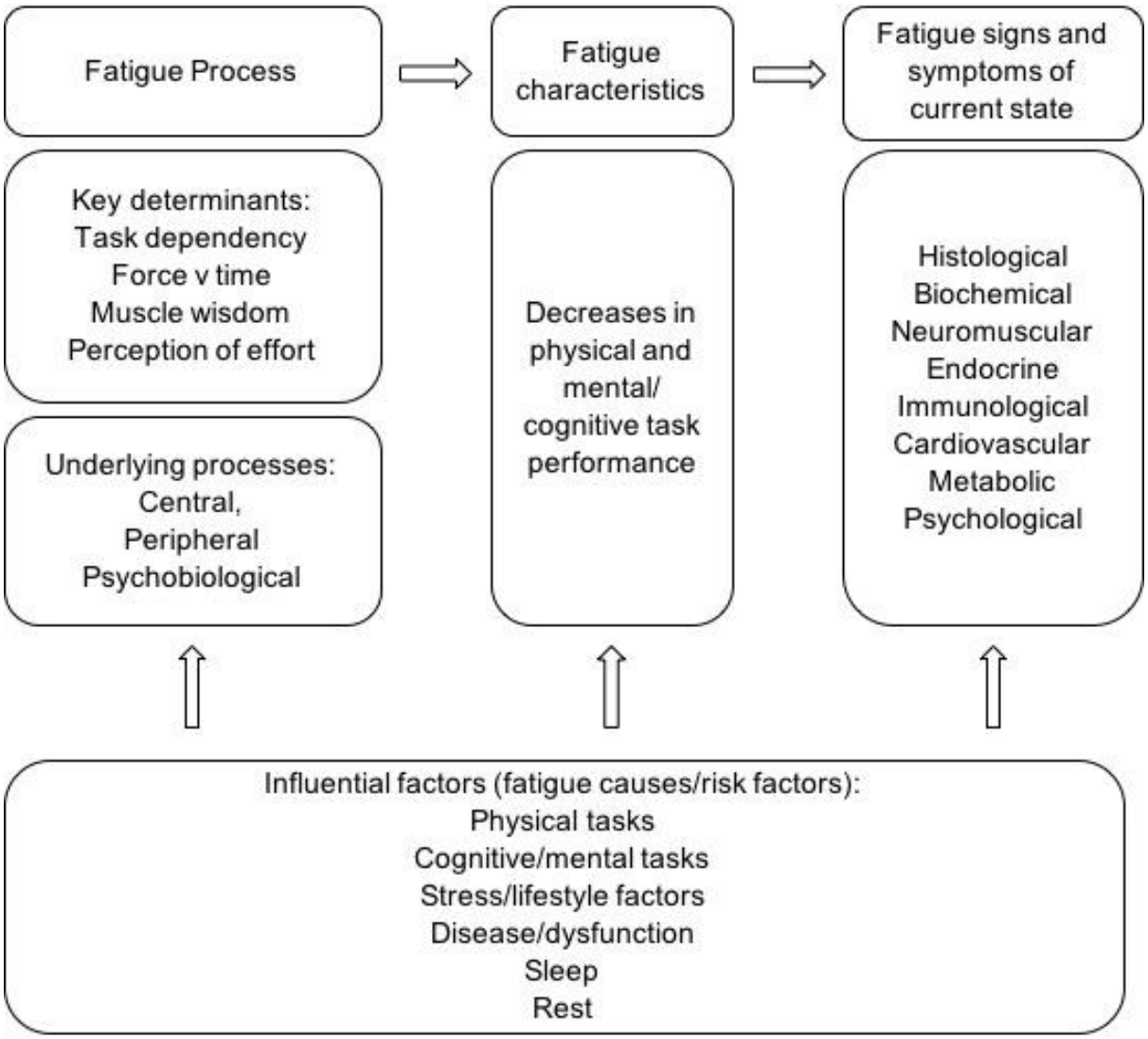

Figure 2.1: A suggestive model of the constructs of fatigue.

2.3 Biological monitoring of athlete status

The monitoring of performance, recovery, and athlete status in real-world environments has become a focal point within the sport science arena over the past 10-15 years. Increasing sums of investment within professional team sports such as rugby and football, and elite individual sports such as cycling have driven the overall desire to train and create gold medal and championship winning athletes (Jeukendrup & Van Diemen, 1998; Cormack et al., 2008; Johnston et al., 2013; Owen et al., 2015). The key development in this recent time period is the monitoring and prevention of excessive fatigue and injury and are therefore critical goals of any sport science and

sports medicine practitioner in order to drive performance gains (Burgess, 2017; Lee et al., 2017). This timeframe is somewhat smaller in comparison to the broader research context of sports science, where the past 30 years has seen many developments in the methods used to measure performance and biological characteristics of athletes. As data collection methods become more accessible to professional teams and athletes either via financial means or development of portable technology, it becomes ever more important that methods that are used in the field are as reliable as possible. As ever, even within a laboratory environment, the variable nature of the human being creates normative ranges for any measured population, and therefore what becomes increasingly important is markers are not used singularly, but rather in combination to gain a broader picture of individual responses and accountability for changes in an athlete's status (Twist & Highton, 2013).

2.3.1 Biomarkers

A biomarker is defined by the National Institute of Health (NIH) as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" (Atkinson et al., 2001). Biomarkers range across biochemical, endocrinological, haematological factors in order to provide a picture of health status, moreover, in the context of exercise and sport performance monitoring, the recovery from fatiguing events and readiness to train or compete (Lee et al., 2017). However, a single measurement of a biomarker does not allow for precise determination of an individual's health status. For example, a measurement of cortisol alone, cannot confirm whether the body is in a relative state of stress since the relationship between that and testosterone have some antagonistic counterbalancing, not to mention the psychological influences upon the stress response that cortisol represents. As such, the testosterone/cortisol ratio gives a more sensitive measure of stress than either measure alone (Lee et al., 2017). Essentially, a biomarker measurement provides a quantitative, individualistic snapshot of a homeostatic response at a certain time point (Lindsay & Costello, 2017). Therefore, the regular collection of the same biomarker is also required in order to observe the trend of an individual's homeostatic rhythms in order to define their current biological state (Lee et al., 2017). Furthermore, the diurnal variation of a biomarker should also be considered.

2.3.2 Use of blood and saliva to measure biomarkers

Biomarkers can be obtained via invasive and non-invasive methods i.e., whether or not the breakage of skin or contact with mucosa or internal body cavity beyond a natural body orifice has occurred (O'Toole, 2012). Typically, blood samples are used to obtain a broad range of biomarkers which are available in the whole blood itself, or within its constituents of serum or plasma. However, the invasive nature of blood sampling can create some logistical issues, from the required qualified personnel being on site (e.g., doctor or phlebotomist), the requirement for a safe, clean environment with appropriate sample collection resources (laboratory, or medical room), and the handling of samples in storage (access to storage freezers, or transportation). The co-ordination of all of these aspects with athlete scheduling becomes a difficult challenge, particularly in team sports where narrow time windows for sample collection (e.g., before or following training sessions) may limit the number of samples available or disgruntle volunteer participants which may influence future adherence. Also, the expense and/or access to the specialist equipment and the time taken to measure can sometimes deter the use of blood sampling, particularly via venepuncture (Burgess, 2017).

Fingertip blood sampling using whole blood can offer a reliable, accessible and cost-effective alternative method for measuring biomarkers. Creatine Kinase (CK) is one such marker and is used as an indirect biochemical indicator of muscle damage through its appearance in the blood when muscle cell disruption occurs. Despite this, the use of CK must be appropriately contextualised when considering its use as a recovery marker, since its appearance in the blood may not be directly consistent with the integrity of muscle cell structure and muscle function (Tavares et al., 2017). This has been shown when analysed concurrently with muscle performance such as maximal muscle contractions (MIVC) and jump performance (Twist et al., 2012). As such, individual variability (e.g. age, gender, training status) must also be considered when interpreting CK responses (Kim and Lee, 2015).

Knoblauch et al. (2010) have evidenced high consistency between fingertip samples taken immediately after venepuncture in 15 untrained students, where samples were collected 15 min before, and 24, 48, 72, and 96 h after eccentric muscle damaging

exercise. A correlation of r = 0.997 was reported, with capillary and venous measures being within 12 % of one another across a wide range of CK values (50 < 5000 U/L). Whilst not strictly showing agreement of CK concentration between the measurement sites, the temporal change in CK appears consistent. The researchers utilised a method of rate-assay spectrophotometry to analyse both measures, where adequate volumes of blood (~5 mL and 250 μL from the anti-cubital vein and index fingertip, respectively) were taken from each source to obtain sufficient samples. Again, the practicality and adherence of these sampling methods may hinder access to athlete populations. Colorimetric assay procedures provide an alternative, ecologically valid solution whereby ~30 μL of whole blood can be taken from the fingertip, applied to a test strip and inserted into a colorimetric analyser such as a Reflotron (Bio Stat Ltd. Stockport, UK). Many research studies have used this form of automated analysis with sports populations due its accessibility, simple and quick sampling method which provides minimal disruption to an athlete's daily schedule (Neubauer et al., 2008; McLellan et al., 2010; Twist and Sykes, 2011). Most importantly, reported coefficients of variation (CV, %) using this measure have been noted at 3.0 % using a Reflotron (Neubauer et al., 2008; Howatson et al., 2012). There are however, discrepancies in CV across some studies measuring the test-retest variation of this particular CK biomarker, with some studies reporting 9 % (Chrismas et al., 2018) and 18.5 % (Harper et al., 2016). Differences in sample technique (venepuncture *vs* capillary), and analysis method (Reflotron *vs* assay) appear to open potential issues of technical error, and difficulties in sampling timings as dictated by the time taken to acquire the sample. In addition, Chrismas et al. (2018) also argue the notion that the variation of participants used in these studies i.e., a more homogenous group such as similarly trained elite athletes (Howatson et al., 2012) versus a more heterogenous or lesser trained group (Chrismas et al., 2018; Harper et al., 2016) could potentially influence the varied nature of the data, with tighter CVs related to more homogenous groups.

Saliva sampling is another non-invasive sampling method which has recently generated popularity amongst researchers and practitioners in sport due to its ease of accessibility within the sports field, and potential for analysis of a variety of biomarkers (Lindsay and Costello, 2017; Papacosta and Nassis, 2011). Human saliva contains an abundance of hormones, proteins, enzymes and microorganisms within its watery

composition (Hassaneen and Maron, 2017). Such salivary constituents can be detected through numerous methods, such as fibre-optic-based detection, high-performance liquid chromatography, radioimmunoassay, high-resolution mass spectrometry, and polymerase chain reaction analyses (Kaczor-Urbanowicz et al., 2017).

Enzyme-linked immunosorbent assay (ELISA) which uses specific protein-based test kits and microplate reading, has traditionally been considered the 'gold standard' procedure for hormone analysis (Aydin, 2015; Coad et al., 2015). ELISA methods are valid and reliable for use with salivary samples however, whilst the principles of salivary collection and analysis are straightforward, there are many considerations which should be controlled for in order to optimise the whole methodological approach. Saliva secretion and regulation is strongly affected by neural control of the autonomic nervous system, which indirectly affects salivary flow rates i.e. parasympathetic activity increases flow rate, while sympathetic stimulation decreases the flow rate (Papacosta and Nassis, 2011). Table 2.1, below, highlights key factors to be adhered to in order to minimise error variance and chances of methodological errors.

Research standards using ELISA techniques usually require samples to be analysed at least via duplicate methods, with intra-assay coefficients of variation appropriately reported for the assay in use. In assaying of cortisol, Calvi et al. (2017) suggest that a ceiling CV of 15 % and absolute difference of 0.03 µg/dl between duplicates be used to establish whether a repeat analysis of samples above these values should be performed. Assay procedures should utilise standards and controls to ensure validity and precision of each assay performed. This reduces the possibility of single rogue samples within the microplate creating large amounts of measurement error and maintains internal laboratory control when drawing data from saliva samples (Calvi et al., 2017).

Measurements of cortisol and testosterone via salivary analysis can provide reference points for blood levels of these hormones. Significant correlations have been reported between blood and saliva for cortisol at rest ($r = 0.89$), during ($r = 0.86$), and post-exercise ($r = 0.93$) indicating that a high level of precision is evident when using either sampling method (Papacosta and Nassis, 2011). However, it is recognised that

serum- or plasma-based samples contain the total concentration of cortisol or testosterone, and not solely the biologically active portion as in the case of saliva. Furthermore, transfer of cortisol from blood to saliva takes 2-3 minutes. As such, this difference in rate of appearance should be considered where narrow time frames between tasks are relevant during any sample collection procedure. Positive correlations for testosterone concentrations are somewhat more varied within the literature, ranging from strong (r = 0.96; Obminski and Stupnicki, 1997), to weak (r = 0.25; Cadore et al., 2008). Crewther et al. (2010) reported moderate correlations (r = 0.73) following a 30 s Wingate test, whereas Cadore et al., (2008) observed their values post-resistance exercise protocol, both using untrained populations. Obminski and Stupnicki (1997) used triathletes and karate athletes in their analysis, and so, once again, the discrepancies appear to stem from homogenous and heterogenous groups being varied within these studies, with the highest reported correlation coming from athlete populations. Crewther et al. (2010) suggests that individual variation in response to exercise i.e. differing adaptive signalling, or protein binding responses and dissociation rates in the blood between regular healthy individuals may be the leading attributable factors in this variation of findings. Nevertheless, the use of salivary testosterone, when sampled regularly and stringently following the described collection guidelines (Table 2.1) still continues to be advocated as a plausible and reliable method in monitoring androgenic biological behaviour in athletes (McLellan et al., 2010; Crewther et al., 2018). Further suggestions of good practice which enhance the profiling of endocrinological behaviour are sampling before and multiple times after competitive events, sampling before and after interventions, sampling at similar time points where possible, and the establishment of baselines to observe individual variability. As mentioned, the consideration of a real-world perspective in terms of accounting for wider factors which may influence marker variability, should be employed (Lee et al., 2017).

Table 2.1. Saliva sample collection: methodological guidelines (adapted from Papacosta and Nassis, 2011).

Factor	Impact or consideration
Timing	Accounting for circadian rhythms of measured hormones.
Exercise	Avoided at least 2 h prior to sampling.
Pre-wash of mouth	Water solution, at least 10 min before collection.
Food/drink	Food/drink avoided at least 2 h prior to sampling. Stimulation of salivary flow; compromised antibody-antigen binding and enzyme activity leading to invalid immunoassay results.
Blood leakage/bleeding	Risk of contamination of saliva – increased amounts of steroid released into saliva from blood.
Stimulation of salivary flow	Avoidance of use of gum or other stimulants of saliva flow – some saliva components are affected e.g. testosterone concentrations are elevated.
Storage	Bacterial growth will degrade saliva samples if stored at room temperature for over 6 h. Storage should be through the use of a freezer between -20 °C and -80 °C as soon as possible after collection and thawed when necessary for immediate analysis.

2.4 Fatigue signs and symptoms and time course of recovery

The range of categories of signs and symptoms as suggested in Figure 2.1 have attracted many studies in order to investigate the identification of a fatigue state, and hence observe the restoration of fatigue towards a “recovered” state. In other words, clarifying the varied human system responses to fatigue and resultant homeostatic mechanisms. Each category demonstrates a separate mechanism of response and development of signs and symptoms. However, a range of mechanisms occur simultaneously depending upon the key determinants and initial processes of the fatigue state. As suggested in Figure 2.1 task dependency and muscle-force relationships are key influencers upon the extent and nature of fatigue processes and signs and symptoms, and therefore a broad scope of literature must be reviewed to contextualise the concepts.

2.4.1 Neuromuscular fatigue

Signs of neuromuscular fatigue primarily has its determinants originating from central and peripheral sources and typically manifests as a reduction in maximal voluntary contraction force (Froyd et al., 2016). This is directly due to a combination of changes along the motor pathways, from centrally in the brain, to locally at the muscle cell (Thomas et al., 2015). Supraspinal modulations involve suboptimal outputs from the motor cortex when encountering an isometric MVC task or prolonged submaximal muscle contractions. Typically, this is measured *via* neurological means, such as stimulated muscle motor nerve characteristics (either pre-neuromuscular junction for central mechanism representation, or muscle stimulation post-neuromuscular junction for peripheral mechanism representation) and their deterioration of discharge during or after an activity. This deterioration is mainly due to an autonomic afferent barrage in the dorsal horn of the spinal cord, relaying to varying sites in the central nervous system. This is achieved *via* group III and IV muscle afferents, who detect biochemical changes from their respective locations within skeletal muscle tissue alongside their homeostatic role within cardiovascular, haemodynamic and ventilatory adjustments during exercise (Amann et al., 2015). Therefore, peripheral changes in skeletal muscle, for example a build-up of metabolites and Ca^{2+} sensitivity increase the demand of oxygen to maintain force output and so the working muscles are compensated via the adjustments named above. The detection of peripheral fatigue can encompass a variety of measures, from localised muscle stimulatory characteristics (M-waves, muscle twitch potentials, to functional movement output measures (power, strength etc.) and reflect states of high and low frequency fatigue. High frequency fatigue relates to the interrupted propagation of the action potential at the sarcolemma, measured through high frequencies of muscle stimulation e.g. 100 Hz, whereas low frequency fatigue represents disruption of the excitation-contraction coupling mechanisms of muscle tissue measured via low frequency muscle stimulation e.g. 20 Hz (Jones, 1996).

There is a plethora of applied research in this field, where the extent of central and peripheral influences varies according to the task in hand i.e., task dependency. Thomas et al. (2015) observed that during cycling time trials of three differing distances using trained cyclists, the neuromuscular fatigue mechanisms varied accordingly. Shorter (4 km) time-trials displayed increased peripheral fatigue

measures such as pre-post exercise quadriceps twitch potentiation versus longer (20 and 40 km) time-trials. Conversely, the longer time trials showed increased central fatigue mechanisms *via* greater reduced motor nerve voluntary activation. Participants were able to self-pace the time trials, however no influence of this was evident in the observed differences in fatigue level likely due to the experience and consistently of the trained cyclists. Since the intensity of exercise (mean power in Watts) and exercise time were distinctly different across the three trials, it is clear that short duration with high intensity output has greater influence upon peripheral mechanisms. In support of more central mechanism occurring through longer duration tasks such as distance running, Place et al. (2004) observed a greater decline in MVC and decreased voluntary activation of the vastus lateralis muscle towards the end of, and 30 min following five h of continuous running. Contractile properties were unchanged, and so, central mechanisms were attributed to the changes shown. However, from the perspective of assessing the neuromuscular fatigue responses at different intensities within an isolated muscle, Gauche et al. (2009) examined eccentric biceps brachii muscle isokinetic contractions at 80 and 40 % MVC (although equal volumes of overall work) upon the pre, post, and post-48 h changes in twitch torque, twitch torque development and relaxation. The authors concluded that central and peripheral mechanisms were similarly responsible for the production of a fatigue state. In context, both trials are very short duration activities, and locally focussed upon an isolated muscle. Therefore, a greater motor unit demand could be the limiting issue in comparison to more functional and co-ordinated tasks such as cycling and running. The determinant, 'muscle wisdom' i.e., the synchronising of the discharge rate of motor nerves as motor unit fatigue sets in (Barry & Enoka, 2007), may have greater influence during tasks that utilise multiple muscle groups, and consequently, more focal central fatigue mechanisms may be offset until later into a functional task.

Long duration intermittent exercise activities show similar responses to prolonged running and cycling, i.e., greater central than peripheral fatigue mechanisms, although to lesser extents of change. During three h of competitive tennis playing by national level players, Girard et al. (2008) measured a 10-13 % decrement in knee extensor MVC in the final part of the task which was also accompanied by a reduction of low frequency stimulation response of the femoral nerve. M-wave amplitude was unchanged, which, together, suggests that more central mechanisms than peripheral

were responsible for the decline in MVC. The authors do also suggest that due to the nature of the game involving many eccentric stretch-shorten cycles at a frequently high intensity, the peripheral contribution may be due to local muscle damage as opposed to metabolic changes in the muscle fibres. Furthermore, the intermittent nature of the task allowed a controlled maintenance of metabolic status, hydration and energy balance – akin to the real nature of tennis. It is therefore likely that as demands upon the peripheral musculature continue for overall longer durations, and for more prolonged bouts, an increased number of factors begin to accumulate an abundance of afferent feedback to the central nervous system, such as increased oxygen demand, temperature regulation, blood volume and hydration, glycogen use and muscle synchronisation strategies and associated proprioception. As a result, central regulation control is the likely fatigue determinant during prolonged activities, and when intermittent breaks are afforded, the relative increase in intensity of activity may give rise to increased peripheral mechanisms. Similar responses were shown during a simulated 120 min soccer match by Goodall et al. (2017) where a combination of peripheral and central fatigue was evident, but ultimately the latter stages of the protocol were synonymous with greater evidence of central fatigue.

Amann (2011) proposed the existence of an 'individual critical threshold' which attempts to explain the functioning of the feedback loop which regulates and restricts the development of exercise-induced peripheral fatigue mechanisms to a particular threshold, or in practice, voluntary termination/exhaustion during an exercise. This is ultimately controlled by the central motor drive (CMD); a reduced CMD would therefore be the fundamental factor as to when the effects of neuromuscular fatigue affect the continuation of endurance exercise. The study by Amann and Dempsey (2008) underpinned this proposed hypothesis through the use of comparative pre-fatiguing protocols on 5 km time trials. The pre-time trial protocols induced states of 'severe', 'moderate' and no peripheral fatigue, with a four min break before the 5 km time trial, and the final states of fatigue were assessed. The authors observed very similar states of CMD at the end of each trial, which suggests that no matter the status of peripheral fatigue in a preceding task, the regulator of performance will always be centrally driven.

A study by Froyd et al. (2016) challenged this hypothesis to some degree in showing that no common critical threshold existed during post-fatiguing exercise of different durations (3, 10 and 40 min). However, the exercise used here was isolated, isokinetic concentric knee extensor exercises, with the designated sets of 15 contractions self-paced across the time frames. The authors did demonstrate similar characteristics in that the longer protocols reflected greater contribution of central fatigue, whilst the short duration displayed greater peripheral fatigue, and therefore concluded that the level of peripheral fatigue is dependent upon intensity and duration of exercise rather than a being limited to a critical threshold (Froyd et al., 2016).

The two points of view describe above are driven from two modes of exercise, once again, emphasising 'task dependency', 'force required over time', and 'muscle wisdom' as the key determinants of the fatigue processes and mechanisms. However, there are distinct differences in the protocols used which may explain the disagreement surround the individual threshold. It appears that mode of exercise is a fundamental influence, as is the complexity of exercise. 'Simpler' (or isolated in the case of Froyd et al., 2016) exercises may bring greater focus to the contractile level of muscle function and peripheral fatigue process, where any given rest periods may dampen afferent feedback driven to the central nervous system. Motor unit synchronisation has been shown to change during isolated fatiguing exercise within the tested muscles (Contessa et al., 2009; Stock et al., 2012). However, functional or multi-joint tasks may broaden the recruitment strategy options and therefore challenging the CMD further whilst somewhat removing the onus on the peripheral fatigue mechanism in a single muscle (Taylor et al., 2016). Furthermore, Stock et al. (2012) observed an increase in fatigue levels and motor unit alterations of vastus lateralis more than vastus medialis, ultimately due to different fibre properties across the two muscles. It was also evident that there was variation within the participant group in this study from a fibre type distribution perspective, so therefore it would be prudent to ensure that the trained status of study participants is heavily considered. Consequentially, the extent of peripheral fatigue is directly related to the duration and intensity of the task, and the muscle(s) being assessed, and therefore varies accordingly. However, in more functional or whole-body movements such as locomotion or sports activity, the increased barrage of afferent feedback from more broad peripheral sources may have a ceiling point at which CMD *must* regulate muscle function.

2.4.2 The time-of-day effect upon neuromuscular fatigue

The extensive concept of fatigue is discussed in detail in section 2.1, where both short and longer-term characteristics of fatigue are reviewed. However, it is worth noting at this point that the volume of research has much more coverage over short-term neuromuscular fatigue during maximal exercise and is sparse across the broader aspect of fatigue affecting longer term recovery from extended physical tasks e.g., after team sport matches (Chtourou et al., 2013). The effects of time of day upon immediate fatigue characteristics are inconclusive, where, in some cases, it has been observed that there is a greater drop-off in muscle power and strength measured via repeated sprints or Wingate tests (i.e., from highest performance to lowest performance), in the evening compared to the morning (Souissi et al., 2010; Chtourou et al., 2012; Souissi et al., 2012). This may be attributed to greater peaks of strength and power reached in evening performances and hence a greater relative fatigue drop-off reached; additionally, the notion that catecholamines also follow a similar time of day rhythm would lead to the observation of higher biomarkers of fatigue and would also be evident following performances in the evening. This reasoning could also be supported through the additional impact that raised lactate and glucose responses during evening performances i.e., a greater anaerobic contribution to energy requirements would suggest that greater overall physiological stress is applied to achieve a heightened performance, and therefore greater levels of short-term fatigue are reached (Chtourou et al., 2013).

Interestingly, heightened maximal aerobic performances have been observed in the early afternoon between 12:00 and 13:00 h (Chin et al., 2015), however, no effects upon the nature of any fatigue characteristics relating to time of day and maximal aerobic performances have been found in the literature.

For regular training of any exercising individual, especially where the adaptations from training and performances are implicit in generating performance improvements, the findings summarised above raise the suggestion that timing of training and planning for competition (which typically is at a fixed time) is vitally important. At the very least, the understanding of time-of-day variation in immediate and acute situations from a

neuromuscular perspective can assist in the programming of training and optimising the desired effects. A recent systematic review by Mirizio et al. (2020) supports the overarching view that optimal neuromuscular performance is affected by the time of day, with the greatest outputs occurring between 16:00 and 20:00 h. Whilst this is largely affected by core body temperature, other influencing factors such as fasting status, environmental exposure pre-exercise, regular training at specific times of the day and active warm-up protocols, can impact the circadian status of skeletal muscle.

2.4.3 Time course responses of signs and symptoms of neuromuscular fatigue

Following fatiguing activity, neuromuscular markers of fatigue have been researched across a variety of study groups, although the time courses studied, and fatigue-inducing activities are highly variable and ultimately display a range of responses relating to neuromuscular fatigue.

Froyd et al. (2013) employed a fatigue protocol involving isokinetic knee extensor and flexor concentric repetitions over a self-paced period (mean 347 s) in completing 30,000 J of work. Knee extensor MVC (52 % of pre-exercise values) and electrical stimulation torque responses (33-68 %) reduced immediately following the muscular work. Recovery of parameters was observed over eight min post exercise, where MVC returned to 74 % of pre-exercise values, and peak torque responses to electrical stimulation varied in their extent of recovery from 60 % for a single stimulation peak, to 85 % for a tetanic stimulation peak. Indices of low frequency peripheral fatigue i.e., low frequency: high frequency peak torque ratio at 10 Hz and 100 Hz recovered to 75 % of pre-exercise values. In all measurements, an immediate recovery response was evident in the initial 1-2 min post exercise, with a gradual change to the values stated above. Albeit within a single muscle group and over a relatively short time frame, overall signs of neuromuscular fatigue (both central and peripheral) are clearly offset in the short term despite an immediate response towards normalisation of neuromuscular properties. Wadden et al. (2012) observed reduced peak twitch force and M-wave levels at least 60 min following fatiguing stretch-shortening cycle (SSC) movements, even demonstrating no difference in response between fast and slow SSC protocols. A similar immediate response to Froyd et al. (2013)'s findings for peak twitch force in the initial 3 min of recovery was observed (70 to 90 %), with a

subsequent decline to approximately 75 % after 60 min. M-wave responses continued to decline to 75-80 % of pre-exercise status after 60 min. In both cases, physically active (>3/4 activity sessions/week) participants were examined, although the protocols in each case postulated different peripheral fatigue mechanisms, with Froyd et al. (2013) suggesting a greater level of low frequency fatigue, and Wadden et al. (2012), high frequency fatigue. The use of faster contractions through SSC activities have also been linked to high rather than lower frequency fatigue previously in the literature (Strojnik & Komi, 1998).

In comparison to more functional actions such as intensive aerobic running (6 km at anaerobic threshold) using trained runners, some similar characteristics were shown by Skof and Strojnik (2006). Low and high frequency twitch torque showed partial recovery to approximately 85% by 60 min post-exercise, and 90 % by 120 min, whereas isometric MVC of knee extensors was still reduced at 60 min (~85 %) but returned to pre-exercise levels through 120-min of recovery. In this case, it appears that central mechanisms were recovered within two h, whereas peripheral mechanisms take longer for this type of activity. Given that the exercise was relatively short term and at a controlled submaximal intensity, the timeframe available to accumulate increased afferent feedback resulting in central detriments may therefore limit the extent of central influence post-exercise, and consequently shifting the focus to more peripheral causes. Furthermore, low frequency fatigue signs appear to take longer to recover than high frequency when high intensity and short duration activities are undertaken (Tomazin et al., 2008; Perrey et al., 2010). Following a high intensity slalom skiing activity in which well-trained skiers completed a 45-gate slalom course in under 45 s, the authors reported signs of high frequency fatigue which normalised within 180 s of cessation of activity (Tomazin et al., 2008). The extent of central (MVC knee extensor torque) and peripheral measures (twitch torque at 20 Hz) in this study changed minimally pre- to post-slalom activity. Although the SSC demand in slalom is very high, the overall workload time is short. Perrey et al. (2010) observed responses pre- and post-12 x 40 m high intensity sprints, which in comparison to the study by Tomazin et al. (2008) would extend the time under load through SSC muscle actions. The authors observed a greater influence of low frequency signs following cessation of the sprint protocol, in conjunction with high frequency responses.

In a more applied field, especially within team sports, the exposure to participants' training sessions on a daily (or more frequent than a single data collection period) basis allows the time course of fatigue signs to be monitored over a longer period. However, the measures used to represent neuromuscular fatigue become limited to more functional activities such as jumping or sprinting rather than specific neural stimulation data. After a simulated 90-min soccer match involving rapid SSC activities, decelerations and changes of direction, Thomas et al. (2017) measured a variety of indices over a 72-h period in 15 trained soccer players. Central fatigue signs recovered within 48 h, whilst peripheral signs remain elevated at 72 h, although interestingly, whilst neuromuscular fatigue was evident, readiness to train was not affected despite a significant perceived soreness and fatigue rating at 72 h. Other functional measures displayed a variation of status: countermovement jump (CMJ) performance was not fully restored at 72 h, and reactive strength index was depressed until 72 h into recovery, whereas drop jump (DJ) ground contact time was unchanged through the recovery period. Maximal 10 and 20 m sprint performance had recovered within 24 h post-exercise. Despite the range of responses of functional and neuromuscular performance, the perceived ratings of soreness and fatigue remained high even though readiness to train was not affected upon warming up for each testing session. A potential conflict issue can be raised here, in that within environments where team training is regular, there may be increased external pressure to train even though functional and perceived fatigue measures suggest otherwise, thereby putting the player at a greater risk of slower recovery, and possibly, injury. In individual sports, the participant may have more control to regulate the activity and intensity of training, resulting in a more controlled recovery from fatiguing training tasks. Two studies by a research group (Magalhaes et al., 2010; Silva et al., 2013) using Portuguese soccer players also tracked neuromuscular signs of fatigue across a 72-h period following soccer match play, although with differing outcomes. In their 2010 study participants demonstrated a lengthier recovery in terms of sprint, jump, and strength performances (at least 72-h), whereas participants in the 2013 study showed clear signs of functional recovery after 24-h. The standard of player and time of season are vastly different across these two studies, the former examined 2nd and 3rd division players during a preseason period, the latter, professional players following a competitive season match. This variation suggests that there is likely a factor of conditioning level relative to the standard of athlete and how accustomed they are to match-specific intensities,

and once again reflects the determinant of task dependency on the extent of fatigue (Barry & Enoka, 1997).

Research in rugby league represents a different set of task characteristics since greater upper body demands, coupled with increased forceful collisions are also integrated with high intensity running. Johnston et al. (2015) monitored fatigue states of sub-elite youth rugby league players following match play and observed that upper limb neuromuscular fatigue assessed via bench press performance was maintained for longer than lower limb CMJ and squat performance. Lower limb function returned to pre-match values by 48 h post-match, whilst bench press performance remained elevated at this time point. Twist and colleagues (2012) monitored CMJ performance post-match of elite rugby league players, with division of player position to forwards and backs, since the requirement of each position category displays different activity demands. Backs demonstrated a greater time course of lower limb (*via* CMJ) neuromuscular fatigue than forwards, with forwards returning to pre-match levels by day two post-match. The backs carried a moderate effect size of reduction at the same point, although in relative terms, the mean difference in CMJ flight time at day two was 1 % between the positions. Further recovery time points were not reported. McLellan et al. (2011a) supports these studies' findings for lower limb fatigue signs in showing that peak power *via* CMJ was normalised by 48 h following a competitive rugby league match. Data from rugby union shows similar responses with CMJ performance reduced at 36 h, but fully restored at 60 h post-match following a professional domestic league game (Shearer et al., 2015).

Ultra-distance events which involve a sustained lower intensity of effort across a very extensive duration have demonstrated greater influence of central mechanisms post event for up to 16 d, while peripheral neuromuscular signs were, on average, restored within this period (Millet et al., 2011). In this particular study a mountain marathon (average 37 h of activity) generated broad variation in the recovery data for 22 athletes, with some but not all participants having normalised fatigue signs for knee extensors and ankle plantar flexors within 16 d. This variation would suggest that for events of this nature, a greater influence of individuality for physical condition, biomechanics, and race strategy may generate the potential for a range of responses in terms of the time course for restoration of central and peripheral markers i.e., two

weeks appears to be the norm for normalisation, while some athletes may take longer. Petersen et al. (2007) observed the recovery of eight elite marathon runners post event and also showed a greater extent of central fatigue detriment rather than peripheral. Furthermore, the MVC of plantar flexors was still significantly lower at two days post-race, whereas knee extensors had normalised, and by day five, plantar flexors had also returned to baseline figures. Unfortunately, no other intermediary measures were taken and so the exact time frame for MVC restoration for this muscle group between two and five days is unknown. Nosaka et al. (2010) followed an ironman triathlon athlete case study post-race for 15 d and provide further context to the above studies within long distance events. The authors observed a normalisation of knee extensor MVC, CMJ, and squat jump neuromuscular performance at eight days, with perceived recovery following suit. Only running economy was reduced at day 15 and knee flexor MVC still reduced at day eight. Albeit a single case study, the value of individuality can still provide some degree of insight to the patterns of recovery markers following this type of extreme event.

In summary, the neuromuscular signs of fatigue are typically dictated by the intensity and duration of the fatiguing activity, furthered by individual variation within a participant group. In shorter, more intense activities, high frequency peripheral fatigue appears to cause more immediate declines in neuromuscular function, although this normalises within minutes following activity. For longer duration activities, central fatigue signs are more prevalent and account more for the time course in restoration according to the exercise in question (potentially from 5-16 d). For intermittent high intensity exercise such as team sports, more low frequency peripheral signs appear to influence neuromuscular performance for up to 72 h.

2.4.4 Endocrine responses to activity

The endocrine system provides a fundamental basis of control to the homeostatic balance of hormonal status in the human body. It is inherently linked to the autonomic nervous system in its responses to demanding circumstances. This is to maintain physiological integrity and provide some form of basis of adaptation to all stressor-related information received by the body, and hence is also referred to as the neuroendocrine system (Ulrich-Lai & Herman, 2009; Ball, 2015). The alterations in

endocrine function during or as a result of exercise stress are determined by the intensity of activity, the level of stress induced, and the training status of the individual. The responses are controlled through the sympatho-adrenal sub-system, the hypothalamic-pituitary-adrenal (HPA) axis, and the hypothalamic-pituitary-gonadal (HPG) axis (Ball, 2015).

During exercise, the increase in sympathetic activity of the autonomic nervous system reflects the activity of the motor cortex i.e., the neurological feedback and feedforward of muscular actions. This sensory-motor control drives the release of catecholamines (adrenaline and noradrenaline) from the adrenal medulla in order to assist neural transmission, increase metabolism, and assist the mobilisation of blood glucose through stimulating glycogenolysis and carbohydrate metabolism (Crewther et al., 2011; Ball, 2015).

The HPA axis has a variety of stimulants, ranging from hypoglycaemia, acidosis, alkalosis, and inflammation, as well as psychological stress. The stimulation of the HPA axis results in hypothalamic stimulation *via* corticotropin-releasing hormone (CRH) which directly causes the pituitary gland to produce adrenocorticotropic hormone (ACTH). This, in turn directs the release of cortisol, a glucocorticoid, from the adrenal cortex into the blood circulation (Inder & Wittert, 2008).

The two mechanisms mentioned above, act antagonistically. The former responds as a driver of exercise stress when demands are increased, and the latter as the mediator in order to provide a homeostatic counterbalance as an anti-inflammatory agent. In conjunction with HPA axis and sympathetic adrenal stimulation, the HPG axis provides the means of tissue regeneration following damaging exercise as the body aims to restore a normal environment that was evident pre-exercise.

Essentially, the extent of fatigue, and therefore the demands for recovery from any activity is dependent upon the initial exercise or activity stimulus (Bartolomei et al., 2017). In essence, the body will adopt a more catabolic environment to support the demands of the activity, which in turn creates the extent of anabolic response to ‘recover’ the body. Therefore, it is necessary to review the roles of primary catabolic

and anabolic hormones which have a primary influence upon these two states, in particular, cortisol and testosterone following different forms of activity.

2.4.4.1 Acute cortisol responses to activity

The key role of cortisol (C) during exercise is primarily similar to catecholamines in glycogenolysis and gluconeogenesis, however there are wider influences of C, such as anti-inflammatory response, and immunosuppression (Hackney & Waltz, 2013). The nature of the rise of C is to reflect a state of catabolism and fatigue following physical activity. However, some caution should be taken of this viewpoint, since it is not necessarily a negative or unwanted biological state. As such, there are key roles being played and directed by C in the response to activity. For example, physical and physiological stress from high intensity exercise, or a psychological stress from pre-match anxiety, nervousness or the pressure of optimal performance (Kraemer & Ratamess, 2005).

Under normal circumstances, C displays a circadian rhythm of release (figure 2.2), peaking early in the morning (06:00-08:00 h), and at its lowest in the evening (18:00-20:00 h, Rose et al., 1972; Teo et al., 2011), however, exercise interrupts this normal response by providing a change in homeostasis of the body's physiology and biochemical status. In light of the reduced neuromuscular abilities following exercise as reviewed in the previous section, the elevation of C has a direct association between protein degradation, reduced electrophysiological properties and contractile properties of skeletal muscle post activity (Crewther et al., 2011).

The release of C increases during exercise according to intensity. Hill et al. (2008) observed dramatic increases of C at cycling exercise intensities of 60 and 80 % of $\dot{V}O_2max$ (40 and 83 %, respectively), with no changes at low intensities (e.g., 40 %). Hence there was deemed an intensity threshold of change in line with these findings. Following supramaximal work, Crewther et al. (2010) showed a peak elevation of C 20 min after a Wingate cycle test in trained individuals, although this returned to baseline by 10 min later.

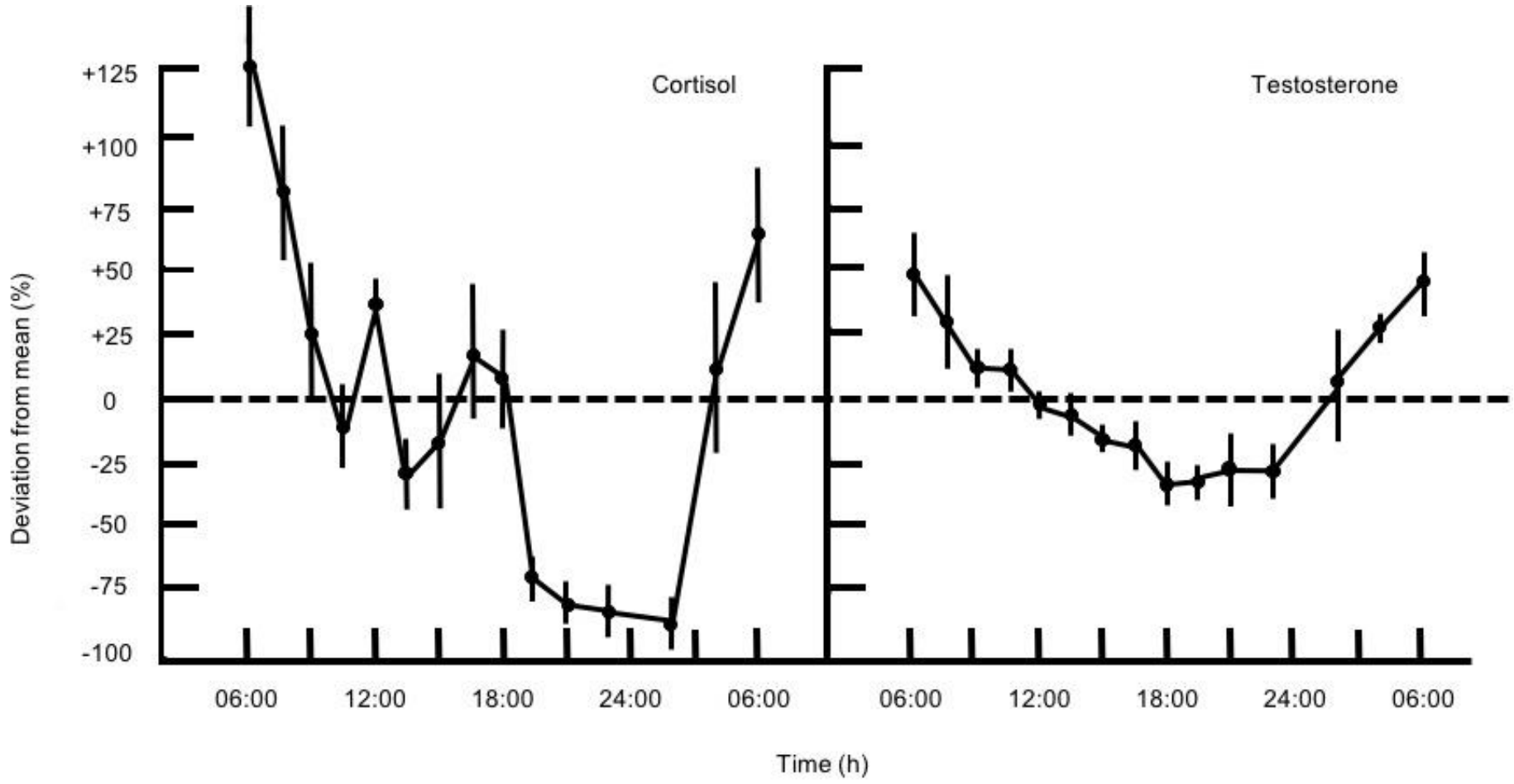

Figure 2.2. Diurnal variations of plasma cortisol and testosterone (Rose et al., 1972).

Caetano Junior et al. (2017) observed a significant (P = 0.022) change in cortisol responses in rugby players following a competitive match, with 30 min pre-match values reported as 0.26 µg/dl and 10 min post-match as 0.41 µg/dl. Likewise, McLellan et al. (2011b) reported elevated levels of C alongside biochemical markers of fatigue following elite rugby league match play. Mean pre-match (24 h) concentrations were reported at 0.37 µg/dl, immediately pre-match at 0.47 µg/dl and post-match levels of 0.79 µg/dl. Endurance exercise such as treadmill running at ventilatory threshold until volitional fatigue also induces elevations of C as a result of the exercise task (Daly et al., 2005). It appears clear that exercise per se will induce a change in C response which is relative to the level of intensity and stress experience by the participant. An early study in 2000 by Lac and Berthon monitored the hormonal profile of runners during and following an endurance relay competition where the participants completed approximately eight repetitions of a median 11 min of running at racing pace. As expected, increased levels of C were observed following the activity, however, this was mainly due to the final two relay bouts being performed at a higher intensity than that of the first six, mainly due to racing tactics.

Interestingly, when the stress and anxiety of a competitive match fixture is removed i.e., in a simulated training match, the C responses tend to be lower in context than in unpredicted and pressurised competitive environments (Moreira et al., 2009). This may suggest that the psychological intensity of the activity is strongly reflected in the C response. There is further support for this notion in the observation of an acute lowering C response in trained rugby players following four resistance exercises (Beaven et al., 2008). The authors of this study justified that a wide variability in C response was mainly due to the potential that this type of athlete may ‘enjoy’ resistance training and hence, not relate the activity as a psychologically stressing situation. Their training status may also dictate the C response with this type of activity and once again, the level of physical stress could be comfortable for a strength-trained athlete. There is, of course, a potential that in both types of activity the threshold of a workload of 60-65 % of $\dot{V}O_2$ max was not consistently sustained and therefore the stimulus for C release via the HPA axis was not sufficient (Hill et al., 2008).

The reported time course of C normalisation appears to be fairly rapid. Lac and Berthon (2000) report normalisation within 12 h following intense activity, and McLellan et al. (2011b) observed a slightly longer time for rugby players to normalise C concentrations at 48 h post-match. In both cases, this remained true even considering diurnal fluctuations of a naturally higher C earlier in the day, and lower during the evening.

2.4.4.2 Acute testosterone responses to activity

The antagonistic hormone of C during the exercise stress response is testosterone. Testosterone (T) is an endogenous androgenic steroidal hormone which is primarily in the Leydig cells of the testes in males, and by the adrenal glands and ovaries in females (De Luccia, 2016). T interacts with androgen receptors within skeletal muscle, and is controlled by the HPG axis, whereby the initiation of the axis is achieved either by direct nervous stimulation of the hypothalamus or feedback inhibition by T (Vingren et al., 2010). Following hypothalamic stimulation, gonadotrophin releasing hormone stimulates the release of luteinizing hormone (LH) from the pituitary gland, and directly influences the release of T, which, similar to C in its circadian rhythm, peaks early in the morning, and reduces through the day (Figure 2.2. Rose et al., 1972; Inder and Wittert, 2008; Teo et al., 2011). Free, unbound T is taken up by tissues in order to bind to androgen receptors, which only then influences and mediates the anabolic process on a tissue level (Vingren et al., 2010). The beneficial effects of T are not restricted to skeletal muscle, however, connective tissues (collagenous-based tissues including bone, ligament, and tendon), and neural tissues also gain a functional benefit from T stimulation. Given that a key action of T is *via* protein re-synthesis, influences upon tissue regeneration, neurotransmitter release, nerve regeneration, increase cell body size, and neural dendrite length/diameter are also evident in the repair process following damaging exercise. Furthermore, upregulated GLUT4 expression increases glycogen synthesis via augmented insulin signalling, and anticatabolic mechanisms are proposed *via* competitive binding of androgens with glucocorticoid receptors which become downregulated as a result (Kraemer et al., 2017).

Given the nature of the role of T for protein synthesis, many studies have observed the changes in T concentration following resistance training protocols on an acute

level. Beaven et al. (2008) applied four different resistance exercise protocols in a professional rugby player group and observed an immediate rise in T from 220 to 250 pg/ml after the training sets (e.g., four separate exercises of 3 x 5, at 85 % 1RM). However, 30 min later the T concentration had normalised. With the exercises and samples collected in the morning, the authors did not collate any sample measures beyond this time point, so any further temporal change could not be noted through the day. Kraemer et al. (2001) reported no influence of acute bouts of resistance training upon the circadian rhythm of T concentration in experienced weight-trained male participants. Although interestingly, during the resistance exercise protocol a decrease from the awakening baseline in T was noted, before a subsequent increase back to baseline levels again. The T concentrations did not elevate past awakening levels, as suggested in the study by Beaven et al. (2008). A clear difference in the resistance exercise regimes used between the studies Kraemer et al. using 10 exercises, 3 x 10RM, and Beaven et al. using four exercises, but varied volume ratios from 3 x 5 repetitions at 85 % of 1RM, to 5 x 15 repetitions at 55 % of 1RM. A review by Vingren et al. (2010) emphasised that whilst there was a great depth of research supporting acute increases in T concentration following resistance training, acute training variables should be carefully considered in order to ensure a volume threshold is met. This included exercise selection, order, intensity, volume and rest. Increased metabolic demands which elevates blood lactate usually achieved through limited rest between exercise sets is also a driving factor that elevates T in the short-term post-exercise.

The necessity for overload to instigate rises in T concentration i.e., a stimulus that creates sufficient structural deformity, creates the need for increased HPG signalling to upregulate the repair and adaptive process (Hooper et al., 2017). In sports competition where significant structural damage occurs alongside heavy eccentric loading, (e.g., during rugby union or league match play), there will also be a fundamental necessity for T to assist in the drive to restore tissue homeostasis and create adaptive responses. In these cases, it appears that the T response could reduce or be relatively unchanged immediately post-activity rather than increased due to the high concentrations of cortisol created. McLellan et al. (2010) observed T concentration values in rugby league players at pre-match (assumed as a baseline) - 204 pg/ml, 30 min post-match - 123 pg/ml and then a return to within statistical normal

ranges within 24 h. Furthermore, a statistically non-significant lower mean T concentration was evident through to 72 h post-match. McLean et al. (2010) also observed rugby league players' endocrinology over an extended post-match period, where no significant statistical change in T concentration within the following 5 d post-match were noted. Where a 9-d turnaround was analysed, some T elevation at day 6 was evident, but could be influenced by training practices such as resistance exercise sessions given the longer period between matches. West et al. (2013) revealed significantly lower concentrations of T up to 60 h post rugby union match play, reporting concentration values in a similar range to that of McLellan et al. (2010). Again, the mean of the value at 60 h, although not statistically significant was evidently lower and less variable than baseline measures 36 h pre-match. Elloumi et al. (2003) observed elevated T concentrations post rugby union match play for potentially 6 d, however these values were in comparison to rest day values recorded two months previous to the observations during and after the match. While rest day values are imperative to obtain, they should have some time proximity to the subsequent measures of comparison. The concentrations of T can be altered over extended time periods, particularly if hypertrophic and strength resistance training forms part of an athlete's schedule. Therefore, in this case, the post-match values may actually be similar to those, for example, 36 h prior to the match (as with the schedule of McLellan et al., 2010). The researchers did obtain a 6 h pre-match sample which was not statistically different to the recovery concentrations, but again, the proximity of the measure may not give an accurate reflection of a true baseline. A recent review by Slimani et al. (2018) suggests that official competition reduces pre-to-post match testosterone levels by 44 %, while simulated matches increase pre-to-post levels by 34 %. This suggests that the unpredictable nature and increased psychological demands of competition may have significant influences upon hormonal concentrations.

Taking samples on a daily or periodic basis can give rise to potential inaccuracies since that only 'snapshots' of status are being observed. Also, the time of day of sampling should be carefully considered, as previously discussed. A further consideration should also be the timing of competitive activity – sports competition frequently occurs in the afternoon or evening when T concentrations are naturally declining. It may also be the case that during a rugby game where aggression levels

are high, the T concentration at that particular time may also temporarily elevate this (Hooper et al., 2017). Then, as the overall game stress accumulates with the added psychological pressure to win, the antagonistic effect of the HPA axis and the production of cortisol may dampen the immediate rise in T concentrations as normally seen following resistance exercise. Twist and Highton (2013) also raise the point that over an 8/9-month competitive season of rugby league, the psychological well-being of players can deteriorate, which, as previously discussed, could heavily influence the functioning of a competitive athlete's endocrinology. It therefore appears that when more uncontrolled competitive situations are encountered on a frequent basis, and, where significant levels of repeated structural damage are inherent, the immediate (post-activity) stimulation of the HPG axis may be more challenged. Therefore, the T response may be prolonged or drawn out over a longer time period since 'full' participant recovery to pre-match states are typically longer than post-resistance training sessions.

Endurance exercise sessions involving endurance-trained runners do also give rise to similar T responses to resistance sessions i.e., immediate rises in T concentration post exercise (consisting of 30 min endurance running protocols), with a return to baseline within 60 min (Tanner et al., 2014). There is a notable difference in the observed T concentration with endurance-trained participants (100-130 pg/ml) at rest and post exercise (150-180 pg/ml) when compared to resistance trained, or strength and power-based athletes. The consensus of literature in this comparison i.e., chronic adaptations of T concentrations according to training stimulus explains the difference in resting levels of T between specific populations (Vingren et al., 2010).

In the majority of studies, the individual normalisation of T and C occurs relatively quickly, from 30 min to typically within 24 h post-activity. This may simply be due to the observation of single exercise protocols, where there is no subsequent activity from the participants. In field-based cases where the activity is more unpredictable, stressful as well as high intensity (such as during combative or contact sports), the normalisation may extend a little further to 48 h, especially when some form of training activity follows the initial high intensity stimulus and recovery is monitored for an extended period such as up to three-to-five-days (West et al., 2013).

2.4.4.3 Testosterone:Cortisol (T/C) ratio responses to activity

By viewing the interaction of these important steroidal hormones via a mathematical ratio, a function of anabolic and catabolic status can be inferred and has been used by many researchers over the past 15 – 20 years in assessing training influences upon the body (Cormack et al., 2008; De Luccia, 2016). In relative terms, a decline from a baseline in the mathematics of the ratio is considered a catabolic state, and a positive elevation from a baseline, an anabolic state. Essentially, the determination of the ratio could be dictated by a change in either (or both) hormone concentration value, and it would therefore reflect an aggregated status of protein balance, i.e., re-synthesis or degradation (McLellan et al., 2010). This ratio is therefore more sensitive to identifying a particular state than a singular hormone marker since it is considering the interplay between the HPA and HPG axes. Hayes and colleagues (2012) present a 12-h rhythm of this ratio for a young, physically active male sample and is shown in Figure 2.3. The lowest point in the cycle, and therefore where the most relative catabolic activity is identified, is in the early morning at approximately 08:00 h. Despite a high testosterone level at this time of day, the peak elevation of cortisol appears to dictate the ratio between the two hormones at this point in time, and also in comparison to other points during the cycle. Therefore, it is vital that the relative level of each contributor to the ratio is considered rather than assuming that high levels of a single hormone represents either a catabolic or anabolic status.

It appears to be a consistent observation in the literature that where a fatigue state is reached during an activity, the immediate post-activity status of the T/C ratio is negative, hence, participants are in a catabolic state (Passelergue and Lac, 1999; Elloumi et al., 2003; Cunniffe et al., 2010; McLellan et al., 2010; West et al., 2013; Anderson et al., 2016; Popovic et al., 2019). Thorpe and Sunderland (2012) noted a similar status before and after a competitive football match however, baselines were only taken an hour before match commencement. As discussed previously, baselines taken too close to competition may not provide a true reflection of ‘resting’ status; it is feasible that a pre-competition anticipatory effect on both C and T concentrations would create a more catabolic internal environment than if the marker was taken the previous day (Chatterton et al., 1997; Van Paridon et al., 2017).

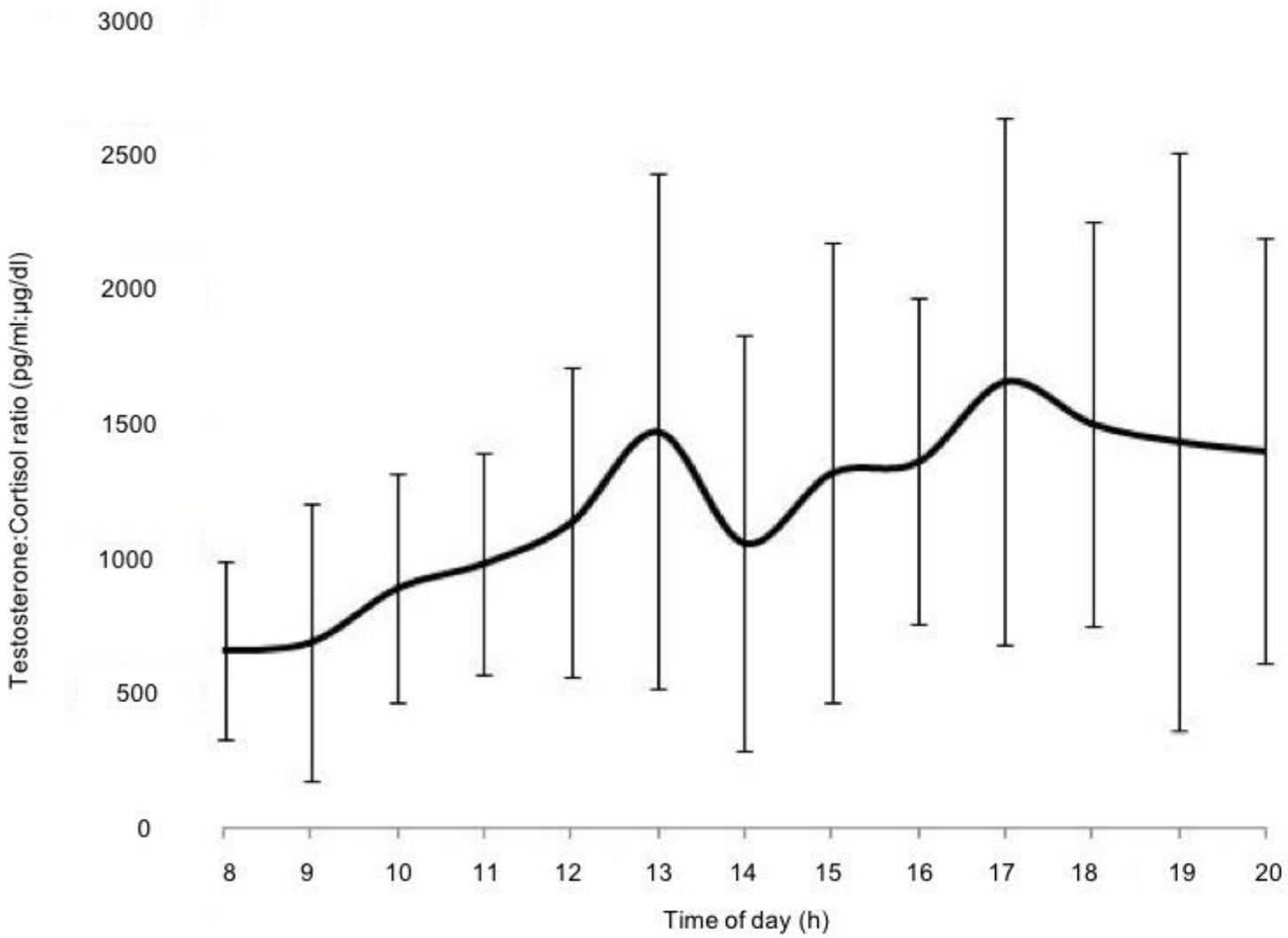

Figure 2.3. Diurnal variations of the salivary testosterone/cortisol ratio (Hayes et al., 2015).

Similarly, Anderson et al. (2016), Cadore et al. (2008) showed that in both strength-trained and untrained populations, the T/C ratio response immediately after a superset resistance training session remained statistically unchanged to that preceding the training session, although the individual C and T responses differed between the two groups. The trained group displayed lessened C and T responses to the untrained group, perhaps due to the requirement of a greater training stimulus than that provided, and the adaptations made from their history of strength training (range 4-25 years). The authors proposed that while the untrained group did display greater C responses, this was offset by an acute T increase potentially due to their lack of experience of resistance training and therefore more acutely sensitive hormonal responses (Cadore et al., 2008). Unfortunately, no further post-exercise measures were taken to observe the adaptive response would have that followed. Nevertheless, a catabolic state following fatiguing activity is an expected response due to the energy requirements to fuel recovery processes and create an anti-inflammatory effect to reduce excessive damage to tissues. However, the time course of normalisation of the T/C ratio appears variable across research studies involving participants engaging in competition.

Anderson et al. (2016) observed an immediate state of catabolism in 12 endurance trained runners following an endurance treadmill run to volitional fatigue at ventilatory threshold. The T/C ratio had normalised at 24 h, although individual values of C and T normalised at 48 and 72 h, respectively. As suggested earlier, the ratio aims to reflect a relative balance between the two hormone profiles, and therefore in this case the authors argue that the T/C ratio is *not* accurately reflective of anabolic:catabolic status, due to extended changes in the HPA and HPG axes as indicated by the C and T responses. Cunniffe et al. (2010) and Ellouimi et al. (2003) both showed a slightly longer ratio normalisation post-rugby match by 38 and 48 h, respectively, at which point, an increase in T/C ratio was observed in comparison to baseline ratios. Passelergue and Lac (1999) observed a similar response in wrestling competitors. McLellan et al. (2010) observed a T/C normalisation by 48 h post rugby match play, which was maintained until the next bout of high intensity training at 120 h post-match, at which point the T/C ratio substantially reduced as a result. West et al. (2013) noted a lowered T/C ratio to baselines for up to 60 h post-rugby union although no measure was taken between 36 and 60 h. Despite this, the mean ratio value reached at 60 h

still did not match that of pre-game baselines, although this was not statistically different.

It appears that in light of the variety of studies utilising a range of activities as the study intervention, the more uncontrolled environments such as real time match-play may induce greater stress-related catabolic states than resistance or endurance tasks within a controlled environment due to the competitive and unpredictable nature of the task (Hayes et al., 2015). Furthermore, in competitive sport environments, there is evidence that longer-term accumulated effects of training and competition can alter the anabolic:catabolic profile of athletes. Handziski et al. (2006) showed a more pronounced example of this greater catabolic state towards the end of a competitive football season, where accumulated training and competitive stresses can have a more chronic effect upon individual athletes. The researchers found higher levels of cortisol both at rest and after exercise during the third phase of a season (towards end of season), with relative lower levels of testosterone compared with earlier points in the season. Ratamess et al. (2013) showed a similar seasonal response in collegiate wrestlers whereby a lowered T/C ratio occurred steadily from the pre-season period, through to the close of the competitive season. Despite a consistent measure of C throughout the sampling period, the gradual reduction of T concentrations was the determinant of an increased catabolic status.

Overall, the endocrine status of an individual can display a useful insight to the basal signalling process that dictate recovery from fatigue, or combat regular training and competition. It is worthy of note that while hormonal status may return to resting or baseline levels relatively quickly following a fatiguing exercise task, other biomarkers that are reflective of muscle healing status should also be utilised to expand the overall view of an individual's recovery from damaging activity.

2.4.5 Biochemical responses to activity

Skeletal muscle-damaging tasks are a major determinant of the extent of post-activity fatigue, where, as previous discussed, the neuromuscular interactions of damaged regions are significantly affected (McKune et al., 2012). Biochemical markers are typically used in conjunction with neuromuscular output tests such as

countermovement jumps or MVC contractions, in order to gain an indirect view of the internal damage caused by intense activity, in particular, the analysis of muscle enzymes such as Creatine Kinase (CK; Ascensao et al., 2008; McLellan et al., 2011a; Johnston et al., 2013; Doeven et al., 2018). Muscle damage is ascribed to mechanical disruption of the fibre, including membrane damage, myofibrillar disruptions, loss of z-disk integrity which have an effect upon excitation-contraction coupling within muscles (Nédélec et al., 2012). As a result, CK is released into the interstitial space and plasma circulation, where concentrations of CK usually peak approximately 24 h after damaging exercise (Lee et al., 2017). From this time point, it appears that the normalisation of CK as an indicator of fatigue caused by muscle damage is varied amongst individuals and across the fatigue-inducing activity. In any case, it is well established the overall homeostatic response to damage-related and fatigue-inducing activity is for the biological systems of the body to adapt to the stimulus provided through increased protein synthesis and tissue remodelling in order to advance the body's system capabilities to coping better with a similar stress (Grobler et al., 2004). Magal and co-workers (2010) observed a broad between-participant variability at the peak of CK concentrations following a single limb, 3 x 50 repetition eccentric protocol on an isokinetic dynamometer. Baseline measures were reported at 146 $\pm$ 32 U/l, while peak elevations ranged from 109-945 U/l (mean 315 $\pm$ 48 U/l). This peak was significantly ($P < 0.05$) elevated at 24 h post-activity, which then remained elevated in comparison to baselines for up to 96 h, although not reaching statistical significance. The authors did not report the actual significance level despite this elevated group mean of between 220-280 U/l value over the 48 – 96 h recovery period and reported significant ($P < 0.05$) muscle soreness ratings *via* a 10-point Likert scale. In line with a consistent argument throughout this review, the heterogenous nature of the participant group is a strong influential factor upon the biological responses. Seventeen untrained college-age male students were used, and so while this represents a regular population, the authors did also record a natural variation in the muscle fibre typing of the participants *via* muscle biopsy. Standard deviations of 3.6 % across percentage distributions of type I (mean 40 %) and type II (mean 60 %) groups allows for a natural mathematic average for upper and lower differences of 27.2 and 12.8 %, respectively, across fibre type distribution. This line of reasoning is furthered by the evidence that predominance of type IIb fibres correlates significantly

(r = 0.58, P < 0.05) with levels of soreness at 48 h post activity. Despite these observations, the actual level of soreness reached only between 5 and 6 on a 10-point Likert scale, potentially suggesting that the damaging activity was influential in some participants more than others. Therefore, alongside the consideration of the population being measured, the appropriate level of stress of the activity should be carefully applied.

Research using trained populations requires more bespoke application of fatigue protocols in order to gain the desire effects upon the stress response. Kristoffersen et al. (2018) recently observed the responses of 12 well-trained cyclists to heavy strength (HS) and short sprint (SS) protocols in a crossover design that replicated training sessions implemented by Norwegian world-class cyclists. Both protocols lasted 45 min and involved repeated maximal efforts (3 x 4, 8 s efforts: SS) or 3 x 6 repetition maximum (RM) for five lower limb resistance exercises. CK levels for both trials immediately rose from baselines (250 U/l) for 30 min, but the HS trial caused significantly elevated CK concentrations across 45 h post activity (P < 0.034), with peak values reaching a mean of 450 U/l.

In contrast of activity duration, Neubauer et al. (2008), observed the damaging effect of Ironman triathlon racing in 42 non-professional, well-trained triathletes. CK increased on average by 1195 % immediately post-race (P < 0.001), with a maximum CK concentration 4316 % above pre-race baseline, which then remained significantly elevated for at least five days (+281 %). The peak concentrations reached over 4000 U/l 24 h post-race, vastly greater than any values reported in shorter duration, high intensity activities for the same time period. This demonstrates that although the intensity is important considering the population being observed, the repetition and duration of stress also plays a vital role. It makes sense that ultra-distance activities will sufficiently challenge the physiological capabilities of an individual despite the overall intensity requirement to complete the distance being relatively low in comparison to heavy lifting. Morton et al. (2009) explains a critical threshold hypothesis whereby the absolute workload, novelty of exercise stress, and necessary fibre recruitment are all accountable towards whether an activity reaches the critical level that causes sufficient adaptive stress in muscle and connective tissues. Naturally, individual characteristics i.e., age, gender, and training status with resting

levels of various cellular components) will also influence the nature of change. In this case of ultra-endurance triathlon racing, the volume (combined intensity and duration) of activity is extremely high, as is the novelty of such events in that athletes will likely only complete this volume of activity on the race day itself, and therefore the sustained effects upon the microstructure of musculoskeletal structures is exacerbated.

Where activity becomes more unpredictable, particularly in competitive team sport environments, and especially where training schedules influence pre-competition levels of cellular status, the variability in monitoring muscle damage becomes highly individualised in terms of baselines and peak concentrations (Nédélec et al., 2012; Twist and Highton, 2013). In soccer, Ascensao et al. (2008) observed the recovery profile following a competitive match for 72 h, reporting significant elevations of CK concentrations throughout the 72-h period. Baseline measures were on average, 200 U/l, with peak values reaching over 800 U/l at 24 h through until 48 h, reducing to 600 U/l at 72 h. Standard error of the mean (SEM) was reported throughout the recovery profile, and in particular, peak concentrations at 48 h was accompanied with a SEM of up 100 U/l for the mean value. At baseline pre-match, the SEM reported was approximately 50 U/l. McLellan et al. (2010) reported a longer profile of recovery following competitive matches for elite rugby players, reporting mean values and standard deviation. Baselines consistent with all previously reviewed studies using trained populations were recorded (256±113 U/l), with peak CK concentrations recorded at 24 h post-match (941±392 U/l), steadily declining to 365±139 U/l at 120 h post-match, still significantly ($P < 0.05$) above baseline values. The standard deviation of the mean appeared to broaden particularly through the period of peak concentration (24–48 h), indicating the varying effect of match play upon each individual on the field. McLellan et al. (2011a) reported significant correlations between CK concentrations following the same rugby match as McLellan et al. (2010) at 24, 48 and 72 h with the number of hit-ups, intensity impact zones (r values from $r = 0.62$ to $r = 0.77$). This implies that the individuals in playing positions that perform greater high impact collision as well as high intensity running bouts i.e., forward positions, will experience greater levels of CK, as a representative of muscle damage from blunt trauma as well as repeated eccentric loading. Ultimately, due to the unpredictable nature of a competitive match, it is more likely that broader variation is observed within this setting

than in laboratory-controlled environments, and therefore the individual recovery responses, in practice, should assist in informing player recovery protocols.

2.5 Effects of whole-body cryotherapy upon human characteristics

Popular modalities include cold-water immersion and whole-body cryotherapy. Cold-water immersion (CWI) has been researched extensively over the past 20 years, where varying extents of benefits and even detrimental effects upon recovery have been reported and discussed (Leeder et al., 2012; Stephens et al., 2017). As a result, the recent review by Stephens et al. (2017) highlights a broad range of methodological variation across the many research studies using CWI, such as immersion depth, water temperature, length and frequency of treatment, participant sampling, outcome measures, and fatigue/performance protocols. The authors concluded that no 'one size-fits-all' protocol exists for CWI and hence the factors mentioned above should be carefully considered in order to establish: (i) the objective of recovery, (ii) the fatiguing and subsequent exercise stimulus, (iii) body temperature at the beginning of immersion, (iv) individualistic physique traits, (v) gender, and (vi) the duration of recovery demands. In light of the abundance of research that has been conducted using CWI, and the resultant practical recommendations stated here, the focus of this thesis warrants greater emphasis upon the lesser-established effects of whole-body cryotherapy.

Whole-body cryotherapy (WBC) is a modality that has gained public attention in recent years through its use by many high-profile professional level teams as suggested by mainstream media reports since 2012 (BBC, 2012, Roberts, 2012; Murtagh, 2017). WBC has therefore become popularised and endorsed as an accessible means of therapy for the non-athletic population as well as athletes with the intention of providing general health benefits (Olsen, 2017). As with many 'non-medical' treatments accessible to the public, whilst the 'benefits' appear to be endorsed by health professionals and athletes alike i.e., akin to the media reports referenced above, the supportive evidence through experimental trials and published literature is much in its infancy in comparison to that of CWI. As such, the effects of WBC require greater depth of investigation in order to appropriately inform practice and application.

Studies using WBC in the clinical setting have been published since the 1970 and 1980s, mainly originating from Japan and Eastern European nations and was originally proposed to assist in reducing pain levels in patients with rheumatoid arthritis (Yamauchi et al., 1981; Taletaviciene et al., 2012). Since this time, the majority of research has been based upon investigating the stressogenic reactions of major body systems to WBC i.e., thermoregulatory, immune, endocrine, cardiovascular, respiratory, and muscular. The effects of WBC on athlete recovery is much less established and little is known about its use practice.

WBC is administered via short duration (2-3 min) exposure to extreme cold air of -110 to -130 °C in temperature-controlled units (Banfi et al., 2010). The units are specifically designed with separate chambers of graded temperatures (e.g., -10 to -60 °C), assisting in accustoming the user to the extreme conditions within the designated treatment chamber at the lowest temperature (Kruger et al., 2015). The user is able to walk into each of the chambers, minimally dressed and therefore the whole of the body is exposed to the conditions (Lombardi et al., 2017). This is considered a very different approach to other methods of cold therapy, for example, localised ice packs, and CWI. An alternative method of 'cryostimulation', also termed partial body cryotherapy (PBC), does expose the body to extreme low temperatures within a semi-enclosed treatment pod. However, the head and face are not contained within the pod itself and therefore the surrounding environmental conditions have the potential to mix with the conditions within the pod. As a result, different temperatures observed in the pod have conflicted with manufacturers' claims (Savic et al., 2013). For this reason, the focus of the following review of literature concerns the use of WBC chamber-based modalities. The systematic review which follows this chapter considers the outcomes across PBC and WBC modalities separately.

2.5.1 Thermal and regulatory responses to whole-body cryotherapy

Westerlund et al. (2003) and Costello et al. (2012) have observed significant thermal responses to WBC in non-athletic participants. Rectal temperatures (as an indicator of core temperature) following WBC exposure have shown a gradual decline after 60 min of 0.4 °C from pre-exposure normal body core temperature measures (37.4 - 37.7

°C). The former study did not note a statistically significant drop in temperature, whereas the latter did so ($P < 0.05$). The key differences between the studies were the exposure time (2- *vs* 4-min exposures, respectively), and the participant groups. It appears that a shorter time period with sedentary female participants may elicit a lesser pronounced effect than in healthy, active males, which suggests that varied responses will be determined by gender and anthropometric stature (e.g., an individual's body fat percentage). Nevertheless, in the periphery, muscle and skin temperatures appear to reduce with greater effect. Westerlund et al. (2003) recorded a mean skin temperature reduction of 19 °C, from 32 to 13 °C immediately after the WBC exposure in their study, with a gradual return to pre-exposure values by 30 min. Costello et al. (2012) provided similar observations of changes in skin temperature, however, with only a 12 °C reduction from baseline values (30 °C). Similar to those reported by Westerlund et al. a return to pre-WBC exposure recordings was reached within 60 min of the exposure. Again, it may be the difference in gender and body stature that influences the rate of heat transfer i.e., the greater the level of subcutaneous fat tissue, the slower the rate of change from the core to the periphery of the body. This gender difference i.e., increased body thermal resistance in females has been previously demonstrated during CWI up to the level of the neck at 18 °C by Tikuisis et al. (2000), and more recently following WBC by Cuttell et al. (2017) and Polidori et al. (2018).

Since Costello et al. (2012) observed no differences between CWI and WBC responses, the reasoning for the differences discussed between studies seem feasible. Costello and colleagues also recorded the response of the vastus lateralis muscle temperature following WBC exposure, this significantly declined from 35.7 to 34.1 °C at a 3 cm intramuscular depth, and 34 to 32.4 °C, at 1 cm. The extent of these reductions continued for up to 60 min, however no further measures were recorded thereafter. It is thought that following the removal of the WBC stimulus, the body experiences a well-known phenomenon, called 'afterdrop'. This is defined as the continued cooling following the removal of cold stress and is likely to occur through two processes (Westerlund et al., 2003). Conductive and convective afterdrop may occur simultaneously, whereby the conductive contribution relates to heat transfer down a thermal gradient from warmer to colder areas, and via convective means

through venous return of cooler blood from the periphery. It is therefore expected that skin cooling and re-heating occurs much more rapidly than core changes since it is immediately exposed to environmental temperatures after the WBC effect is removed. The deeper the region (i.e., deep muscle, and core) would receive greater influence from these delayed afterdrop mechanisms.

Zalewski et al. (2014) reported alterations in parasympathetic functions, in particular, cardiovascular and autonomic nervous system alterations following WBC. The authors concurred with the previously outlined studies with a peak reduction of core temperature at 50-60 min post-WBC exposure, however, also showed a return to baseline measures within 3 h. Immediately post-WBC, a significant decrease in heart rate was observed, perhaps evidenced by activation of the vagus nerve, and an increase in arterial baroreceptor sensitivity resulting from enhanced venous return. Furthermore, a parasympathetic effect was evidenced by the authors since they noted that spectral analyses of heart rate variability and blood pressure variability significantly indicated a demonstrable change following WBC, however, this was only evidenced immediately after WBC exposure. The key mechanism for these changes seems to surround centralisation of blood flow *via* enhanced venous return. This would result in stronger hydrostatic pressure in the vessels of the chest, alongside a lower fluctuation of blood pressure thus resulting in an altered sympathetic:parasympathetic ratio (Zalewski et al., 2014). In support of this evidence of parasympathetic alterations, Hausswirth et al. (2013) also demonstrated norepinephrine release during WBC, a major driver of peripheral vasoconstriction. The authors also observed strong evidence of parasympathetic activity via heart rate variability indices. Whilst norepinephrine release is an immediate sympathetic response, the resultant chain of events shifts to a parasympathetic predominance. Additional stimulation of trigemino-cardiac reflex receptors located in the face may have accentuated the response which further augments vagal output to the heart. As a result, increases in blood pressure and reductions in heart rate triggers the baroreflex and lowers the sympathetic tone of the autonomic nervous system (Hausswirth et al., 2013). Whilst the thermal effects appear short term, the authors propose that the more the body is cooled, the more the ANS is stimulated, with a larger effect upon the parasympathetic tone.

2.5.2 Exercise recovery responses

There is a growing body of literature the describes the effects of WBC upon a varied number of responses following exercise stress, exercise-induced fatigue, and athlete training environments. Typically, a range of biological markers have been utilised in order to observe the effects of WBC utilising elite athletes and health non-athletes, mainly in the areas of physical performance, inflammatory reactions, oxidative stress from exercise, recovery from EMID, endocrine status and blood profiling.

Lombardi et al. (2013) observed a change in haematological profile in 27 national level rugby union players over a summer training camp. Training was on average, for 4 h daily with gym-based exercises in the morning, and high-intensity discontinuous training in the afternoon. Two daily WBC sessions (am and pm) were applied across a 7-d period. The main effects noted were decreases in erythrocyte count and their haemoglobinisation together with a lowered iron status marker, however, given the lack of control group in the study due to all athletes training and undergoing the same interventions the changes observed, it is difficult to establish the real effects of WBC on these markers. Since only pre- and post-camp measures were taken via blood sampling, any inter-session changes were not able to be observed.

Grasso et al. (2014) presented endocrinological markers for the same participant group during the same training camp as described in the previous study. Samples for analysis were collected through salivary means, and therefore an additional time collection point within 10 min after the second WBC session (post-training in the afternoon) was employed in order to assess the immediate effects of WBC post-training. The authors noted a significant reduction of cortisol compared to that in the morning pre-training sample, however, establishing the effect of WBC is difficult since the afternoon reduction follows a normal circadian pattern. However, it is likely that two training sessions of high-intensity work would elevate cortisol levels as reviewed previously, and therefore the application of two exposures to WBC may have had a reductionistic affect upon cortisol. No effects upon testosterone were noted in the short term. Following the seven-day period, however, statistically significant increases in testosterone ($P < 0.01$) and reductions in cortisol ($P < 0.001$) concentrations were observed across the athletes. This generated an elevated T/C ratio as a result

effectively placing the players in more anabolic state following the camp. As explained in relation to the previous paper, the lack of a control measure makes it difficult to establish the effect of WBC, and added to this, broad inter-subject variability was noticeable. If more baseline rhythms were established for studies such as these e.g., during an equivalent period of training with no WBC, then the relative effect within a single subject group could be better estimated.

Galliera et al. (2012) followed a similar study layout with another group of national rugby players, in observing changes in bone remodelling markers. Blood samples were drawn before and after a seven-day period of five days of training (3 h per day). WBC was applied daily for 2 min exposures although it is not clear as to the timing of the WBC sessions. The authors did, however, utilise a control group, and found that a significant osteogenic and anti-inflammatory effect was evident through increases of RANK ligand and osteoprotegerin (OPG) ratios compared to the control group. However, caution should be taken in the interpretation this marker since the variability of circulating OPG may not reflect that within bone tissue, and as such distort the ratio being measured (Vega et al., 2007). Banfi et al. (2009) studied a separate set of biomarkers from the same experimental group and procedures as Galliera et al. (2012) in order to analyse biochemical and immunological markers over the rugby training period. A mediated inflammatory response was indicated by a reduction in inflammatory-responsive interleukins IL-2, IL-8, and an increased expression in anti-inflammatory cytokine IL10. Creatine Kinase (CK) was used as a biochemical marker of muscle cell disruption, whereby reduced CK concentrations were observed following WBC. Across the two studies, an immunostimulatory effect is inferred as a result of frequent, short exposures to extreme cold. However, whilst the changes observed may appear desirable, it is not clear that these provide a positive impact on subsequent performance.

A group of Polish researchers analysed 20 elite level kayakers across a ten-day training cycle with WBC applied three times daily prior to training (one WBC session prior to morning training, and two WBC sessions prior to afternoon training), and another ten-day cycle without WBC to act as a control trial. Oxidative stress markers showed a significant reduction during the WBC trial, as did CK, and also an increase in lysosomal enzyme activity in comparison to the no-WBC cycle (Wozniak et al.

2007a; Wozniak et al., 2007b). The authors propose that prior exposures of WBC on a consistent basis prior to training sessions reduce the antioxidant stress, stabilises the lysosomal membranes within cells, thus potentially reducing the level of cellular damage to muscle fibres induced by training. Wozniak et al. (2013) observed six elite rowers with a similar protocol; a six-day training cycle with WBC applied twice per day (morning before training and afternoon after training), and a six-day cycle without the application of WBC. Measures of oxidative and training stress were taken prior to the training cycles, then at days three and six of each cycle. Their results supported that of the earlier studies using three WBC sessions across the ten-day cycle for kayakers, concluding that the maintenance of a prooxidant: antioxidant balance was being maintained through the cycle. CK concentrations showed no significant alteration in the WBC trial, while concentration levels increased by day three, as did C levels. By using WBC, C concentrations did increase at day six, but this was postulated as a sign of the alleviation and delaying of training stresses.

There have been a number of studies who have investigated the effects of WBC on inflammatory markers and muscle damage across a variety of athlete populations. Ziemann et al. (2012) monitored six high-ranking professional tennis players across a five-day period where WBC was applied twice daily, while six players acted as controls all within a two-week training camp in the professional tennis mid-season break. The aim was, therefore, to assess the effects of WBC upon recovery from the season, given that the frequent competition period likely induced fatiguing effects. A range of markers were analysed before and after the five-day block of treatments via blood sampling, including CK, cortisol, testosterone, leukocyte count, and IL-6. Considerable effect sizes (0.45 – 0.52) were observed for decreases in CK and increases in C and T concentrations in comparison to the control group, while inflammatory cytokines showed a compensatory effect in that tumour necrosis factor (TNF) reduced while IL-6 increased as a result of the WBC. The overall increase in leukocyte count, together with the above noted changes converge to suggest that a systemic effect of WBC, and recovery assistance is evident during a training camp period.

Hausswirth et al. (2011) simulated a trail running race on three occasions using nine highly trained runners, and observed the plasma CK concentrations, %MVC, and wellness scores across three recovery sessions at 1, 24, and 48 h post exercise,

where WBC was compared against passive recovery, and infrared treatment. The trial run involved running at 10 % uphill gradients and 15 % downhill gradients. While increased soreness and a significant rise in CK was confirmed following each trial, no differences in CK concentrations were noted, however, MVC was recovered in the WBC trial but not in the passive trial. Pain and tiredness perception reduced after the first WBC session at 1 h, whereas the other trials brought the same effect at (infrared) or beyond (passive) 48 h. At 24 h, well-being scores were the highest following WBC recovery treatments. Pournot et al. (2011) investigated separate markers from the same subject group, but only compared passive and WBC recovery methods. The exclusion of the infrared recovery protocol in this study allowed 11 participants to be observed across a longer time period following the muscle damage-inducing simulated trail run (including 72 h and 96 h post-activity in addition the time collection points noted above). The authors of the latter study concur with the data provided by Ziemann et al. (2012) in that a reduction in inflammatory cytokines was observed via lowered C-reactive protein and IL-1beta following WBC. Interestingly, IL-6 and IL-10 did elevate and rapidly return to baseline following both recovery trials, and TNF remained unchanged from baseline values which differed to Ziemann et al. findings. This may suggest that the trained runners were well-accustomed to the nature of the simulated run, and furthermore, only low intensity running was performed between trials, potentially negating a cumulative fatiguing effect. This is in opposition to the tennis players who entered the experimental period following frequent competitive tennis match play.

Selfe et al. (2013) provided some support to the post-competition benefits of WBC in a squad of professional rugby league players. The authors compared varying WBC treatment lengths (1 *vs* 2 *vs* 3 min exposures) on the following day post rugby league match play, on three occasions. The 2 min protocol appeared to be the most beneficial treatment length in demonstrating the greatest thermal change and lowest tissue oxygenation levels in the lower limb, showing the centralisation of blood flow. No effects upon IL-6 were noted, however, the potential reasons for this finding were that blood samples *via* venepuncture were taken only 20 min post WBC treatment, and also the fact that the WBC was applied the day following the competitive match. Therefore, any potential effects of WBC may have either been missed through sampling too early, or treatment application too late after activity. A large variation in

IL-6 measures was also observed, showing that within a squad of a variety of playing positions, players are likely to experience different levels of fatigue as a result of unpredictable game play and volume of high intensity events.

Continued competitive play, and regular training can, at times be synonymous with a state of 'overreaching' i.e., accumulated fatigue associated with reduced or sustained performance, but at increased effort levels (Meeusen et al., 2010). Schaal et al. (2014) studied ten international standard female synchronised swimmers across two, 2-week intensified training periods leading up to competition, one with a WBC recovery modality included daily, and one without. The trials were counterbalanced by dividing the swimmers into two groups. Training loads reached 125 % of normal training loads used away from competition, which is a standard increase in load towards competition used by the swimming coaches, and therefore a 'functional overreaching' effect is used prior to competition tapering. The use of WBC resulted in a number of significant observations after the training period in comparison to baseline levels at the start of the experiment. Sleep quality was preserved, fixed speed 200 m interval testing at higher intensities brought subjective perceptions of reduced effort and perceived difficulty, and overall perceptions of fatigue were alleviated in comparison to the non-WBC trial. The authors propose that autonomic regulation *via* post-exercise parasympathetic reactivation may have played a role in preserving sleep parameters, and therefore, improved recovery. It was also mentioned that a potential placebo effect could not be avoided since there is no feasible way of blinding participants to WBC procedures, however, the swimmers had no prior experience and expectations of WBC so the placebo effect is unlikely.

Kruger et al. (2015, 2019) reported contrasting findings of using a 3-min WBC exposure in acute recovery of running performance in eleven well trained endurance runners. The runners performed an initial ramp test followed by a high-intensity interval running session on a 1 % treadmill gradient. A walking recovery protocol acted as a comparison to WBC and following this another ramp test was performed to assess acute recovery characteristics. From a performance perspective (Kruger et al., 2015) WBC improved acute recovery as the authors observed reduced cardiorespiratory and perceptual load during the second ramp test *via* lowered heart rate, reduced

submaximal $\dot{V}O_2$ measures and RPE in comparison to the walking recovery trial. Despite these physiological and perceptual differences, in 2019, Kruger et al. confirmed a lack of inflammatory, endocrine, and biochemical alteration caused by WBC that emerged from the whole study. Some reasoning could be attributed to the small sample size (creating potential type II error), and also the accustomed training level of the participant group may not have been susceptible to tissue damage through the protocol, for example no downhill running activity was included which had been previously shown to induce EMID in trained participants (Hausswirth et al., 2011). In addition, only a 24-h post-trial period was observed, and as previously outlined, fatigue characteristics may still be evident after 48 h post-activity (Ascensao et al., 2008).

Not all literature has supported the use of WBC application for recovery benefits. Costello et al. (2012) observed a lack of effect of WBC in untrained participants following an EMID protocol, and also established that joint proprioception at the knee during joint repositioning tasks was also unaffected. Similar to the study by Selfe et al. (2013), the WBC treatment was applied 24 h after the muscle damaging protocol, and therefore the window of potential effectiveness may have been missed in providing cellular benefits as shown in most other studies when WBC was applied immediately after an activity protocol. Russell et al. (2017) also reported a lack of effects from WBC in professional soccer male academy players. Fourteen players were observed in a crossover design comparing WBC treatment with a standard recovery control trial following 15 x 30 m timed sprints. Main effects were observed for 24 h post activity, however, no differences between trials were noted for muscle soreness indicated by CK concentrations, peak power performance output (post-sprint set) or perceived ratings of soreness. The only difference noted was a significant rise in testosterone levels at 2 and 24 h post-exercise following the WBC trial potentially indicating a shift towards earlier protein synthesis signalling. The T/C ratio was elevated at 2 and 24 h in comparison to the control trail but was not statistically significant and therefore it cannot be implied that a relative anabolic state was brought about. It may be the case that measurements beyond 24 h were required to observe the longer-term benefits of WBC upon recovery, since it has been noted that recovery from some muscle-damaging protocols or competitive match play may take up to 96 h (McLellan et al., 2010).

Overall, it appears that a range of factors may influence the effectiveness of WBC upon exercising individuals. Firstly, the training status of participants is a vital factor; the evidence suggests that trained individuals provide a more homogenous sample group in endurance sports, and experience more consistent effects from WBC so long as the fatigue protocol is sufficiently applied. Secondly, where athletes are within a training cycle, there may be more accumulated effects from training that allow WBC to optimise a greater effect. Essentially, the greater the stress upon the individual from both a physical and psychological perspective, it appears that it benefits are more likely instigated by WBC. Where team sports are utilised, the research is very limited, and no conclusive effects can be drawn despite the evidence that greater competitive stresses are much more likely in unpredictable environments. Thirdly, the timing of WBC post activity requires consideration. The only papers supporting a lack of effect from WBC involved a delay in WBC application. All other studies utilised an immediate or repeated application of WBC before or after activity. Finally, despite an indication of sleep benefits, parasympathetic activity and endocrine responses, the impact of when WBC is applied and its perceived benefits upon sleep quality and recovery have also not been investigated.

2.5.3 Current perceptions and practice of whole-body cryotherapy in athlete environments

Whilst the growing number of studies involving WBC is promising, the extent of implementation of WBC for recovery purposes, particularly in team sport environments, is unknown. Tavares et al. (2017) published a review of the effectiveness of different recovery modalities across both codes of rugby, reporting just one study involving WBC (Banfi et al., 2009). As such the rate and manner which WBC research is translating into recovery practices requires clarification. A number of studies have explored the use and perceptions of recovery modalities in a range of team sport athletes over the past ten years in countries such as the USA, Australia and South Africa (Simjanovic et al., 2009; Van Wyk and Lambert, 2009; Venter, 2014; Crowther et al., 2017; Murray et al., 2018). From this body of survey-based research, it is apparent that practices such as cold-water immersion, active recovery, sleep, stretching, hydration, and massage are the most common strategies reportedly used

by athletes. Despite the recent publication of some of these studies, WBC is not stated as a modality used in team sports despite anecdotal reports of elite team sport athletes using WBC for recovery existing in the mainstream media since 2012 (BBC, 2012). As such, the level of implementation, perceptions and beliefs of WBC in practice remain at large, and anecdotal at best. A consensus still exists that transfer of sport science knowledge and research into recovery and injury prevention practices is hindered by many factors such as knowledge, relevance, access, and integration (Martindale and Nash, 2013; Barden et al., 2021). Barden et al. (2021) recently emphasised that little information exists regarding the adoption, implementation and sustained use of practice interventions in both codes of rugby, whilst the research for intervention *effectiveness* is much high in comparison. This disparity is also reflected in the field of WBC, where research exploring its effectiveness shows the potential to be beneficial for recovering athletes, but information regarding implementation and practice is either low or unknown. As such, one aspect of this thesis aims to provide more insight to the perceptions and practice of WBC in team sports, so that knowledge regarding its effects can better influence and inform its use.

2.6 Summary

The complex nature of fatigue incorporates a variety of influencing factors which interact simultaneously and either diminishes the performance of a physical activity or increases the perceived effort to maintain a level of performance. These factors cover local and systemic mechanisms through physical, physiological, endocrinological, chronobiological and psychological means, and hence, have a central and/or peripheral impact upon human function. The overall deterioration of performance or increase in perceived effort is reflective of the culmination of all feedback and feedforward psychobiological pathways, supporting the notion of an Integrative Governor of the body linking the brain and the psyche. The impact of fatigue can endure both short (seconds, minutes) and long (hours, days) term periods dependent upon the level of cumulative stress experienced by the individual. This in turn, impacts the time course in returning to a ‘recovered’ state i.e. when the aforementioned mechanisms reach a pre-activity baseline. The greater the cumulative impact and disturbance of homeostasis of the individual, the longer the timeframe of recovery is

required. As a result, subsequent performance of physical activity is dependent upon the recovery status of the body.

Monitoring fatigue is challenging since a single marker cannot represent multi-faceted interactions. However, the use of a combination of markers can help represent a variety of mechanisms which then can be interpreted to provide a more accurate view of an individual's fatigue or recovery status. The measurement of high intensity physical efforts such as jumping for maximal height, or isometric force outputs provide a direct view of neuromuscular function *via* the central (supraspinal) drive, and therefore a deterioration in these outputs are clearly noticed when central neurological mechanisms are compromised. Repeated high intensity efforts or a long duration of activity instigate the peripheral effects upon structural damage and metabolic changes in skeletal muscle and vascular biochemistry. Blood-derived markers such lactate or creatine kinase can be used to reflect shorter (former) or longer (latter) term disruptions in peripheral tissue composition as a result of repeated or long duration efforts.

Salivary biomarkers can be used to identify endocrine balance, and the careful selection of indicative hormones are useful to appraise a basal level of homeostatic disturbance and cell upregulation in initiating restorative processes. The key markers critically appraised in this review were cortisol and testosterone, which both provide an indication of hypothalamic-pituitary stimulation and then, their simultaneous influence upon either adrenal (cortisol) or gonadal (testosterone) hormone release in males.

The determination of a full 'recovery' from a fatigued state is a challenging concept. While the use of individual markers is useful in representing a specific characteristic of fatigue (such as temporary neuromuscular deficits), more wholistic competitive activities such as long distance running or team sport competition exhibit more broad fatigue signs and symptoms, each with differing time scales of restoration to pre-activity baseline levels. As previously emphasised, the cumulative extent of the *overall* constructs of fatigue (Figure 2.1) will determine baseline function, and therefore the timeframe of recovery. Neurological restoration of maximal voluntary outputs following short duration activities (e.g. up to 10 min) typically occur within minutes of cessation

of the activity. For longer term competition such as 80 – 90-min collision sport fixtures, neuromuscular performance can potentially take between 48 – 72 h. In more extreme events such as ultramarathon or triathlon lasting over 4 – 5 h, it may take up to two weeks to fully restore neuromuscular function due to the extent of induced peripheral damage to muscle and connective tissues. Where peripheral tissue damage is significantly induced, biochemical markers such as creatine kinase may reflect the immediate level cellular disruption. Normalisation of this marker is of use in demonstrating cellular changes, but in light of neuromuscular interactions requiring much more than intact cellular structure, the time course of biochemical restoration is likely overshadowed by continued neuromuscular deficits.

A similar point could be made in regard to endocrine disruption being that cortisol responses are short lived for singular activities and are again relative to the level of stress created. High intensities and duration of activity create increased cortisol responses, however, once sufficient signalling of the cascade of cortisol-driven protective responses occurs, the need for heightened cortisol is reduced. Unless of course, activities such as subsequent stress-inducing training sessions compound the accumulation of fatigue, in which case a repeating cortisol response is ensued. Given that testosterone indicates the anabolic drive in tissue re-synthesis, this is dampened when cortisol concentrations are high. As such, the longer cortisol indicates heightened fatigue and tissue stress, the longer it is before testosterone can produce an anabolic response. Therefore, lower activity stress requiring minimal structural recovery creates a short (within hours) cortisol response and dampened and lesser required testosterone response. The resultant being that the peak and minimal amplitudes of the ratio between cortisol and testosterone are small and short lived, and baseline levels can therefore be reached within hours. Whereas in high damaging contexts such as post rugby competition, catabolic (high relative cortisol, low testosterone) states can be identified for up to 120 h, which influences the time course that tissue re-synthesis and adaptation can occur.

Whilst the understanding of markers of fatigue and their recovery profile is a focal point in this thesis, the review above also highlights the consideration of time of day factors in regards to the timing of activity, measurement, and recovery profile. Fundamental characteristics of human function such as circadian rhythms for endocrinological

release of hormones, neuromuscular outputs, and the requirement for restorative sleep are influential to the recovery process and should be considered.

The challenge of competitive sport is heavily revolved around the ability to train, recover and prepare for the next training stress. The latter part of this review focussed upon the use of whole-body cryotherapy (WBC) and its ability to influence the recovery process using short (2 – 4 min) exposure to extreme cold air (-110 to 135 °C) in bespoke engineered sealed (whole-body) or open-top (partial body) chambers. The scope of research in this field is steadily growing, however, still remains in its infancy in comparison to other recovery modalities such as cold-water immersion. However, there is a clear absence of information describing the implementation of WBC and recovery strategies used by coaches, practitioners and athletes in practice, as well as the beliefs and perceptions around its use. Additionally, the time of day of WBC use in practice is very much unknown and has also not been strictly considered in previous studies either. Therefore, time of day implementation of WBC should be initially explored in current practice and from the perspective personal beliefs and perceptions. This will provide a view of practice on which to base future comparisons of gold standard evidence.

Regarding effectiveness of WBC, a broad variation of markers of recovery such as inflammatory mediators (such as IL-6, IL10, CRP), haematological, biochemical, neuromuscular and perceived markers, have been reviewed in the studies above. The monitoring of endocrine markers in response to WBC is extremely scarce, and thus requires attention. Both laboratory and field study settings are evident, however, the difficulty faced by researchers at present is the design of controlled studies which are comparable across trials in order to corroborate the key changes caused by WBC. This is especially challenging in competitive and/or training environments where the variation in recovery responses is highly dependent upon individual exposure to activity stresses.

However, the current evidence is more consistent in showing reductions in core, muscular and skin temperatures to the point that autonomic parasympathetic responses such as reduced heart rates, vagus nerve stimulation, and increase arterial

baroreceptor sensitivity have demonstrated its thermoregulatory influence. As such, in order to establish how and why WBC may influence recovery and the status of fatigue, it seems logical that this thesis should strategically review studies of a similar nature i.e. post-activity recovery with WBC influence and determine how WBC creates a thermoregulatory influence upon markers of fatigue and recovery. This is intended to help inform and establish the design of controlled experimental studies within a live, high intensity competitive environment such as rugby league, where the demands for recovery at their utmost importance.

Chapter 3

Effects of post-exercise whole-body cryotherapy upon objective and subjective ratings of fatigue: a systematic review.

Introduction

Individuals engaging in physical exercise frequently experience fatigue for a period of time post-activity, especially where sustained high or maximal intensities are reached (Aman, 2011). Therefore, the extent of recovery from physical exercise is a vital factor within any athlete's training or competition regime, since it dictates the performance of subsequent bouts of activity.

The symptoms of fatigue experienced post-activity coincide with perceived descriptors of tissue damage, such as 'stiffness', 'tenderness', 'soreness', and 'aching pain' (St Clair Gibson et al., 2018). In most cases, tissue damage is caused by excessive and repetitive muscle contractions, eccentric contraction micro tearing, or blunt trauma (McLellan et al., 2011). Biochemical markers such as Creatine kinase (CK) have shown to be reliable in indicating an extent of muscle cell membrane disruption through its 'leakage' from damaged tissues into the extracellular environment, whilst others such as interleukins 6 (IL-6) and 10 (IL-10), and C-Reactive Protein (CRP) have been used to observe the expression of pro- and anti-inflammatory response caused by this damage via their respective concentrations (Bruunsgaard, 1997; Vargas & Marino, 2014). Endocrine markers such as cortisol and testosterone have been used to provide a representation of hormonal balance, initially representing the extent of psychobiological stress caused by the activity (McLellan et al., 2011; Crewther et al., 2018). Post-activity, this balance can be used to observe the normalization and basis for protein synthesis as the tissues repair (Russell et al., 2017). From a perspective of neuromuscular function, the restoration of muscle strength, power and co-ordination is observed using physical tasks, for example, maximal voluntary contractions, and maximal countermovement jumping (Twist et al., 2012). Aligned with the biomarkers described, perceived ratings of soreness typically using pressure gauges, Likert or visual-analogue scales have also been utilised to monitor recovery status, and hence the wholistic view of recovery is a vitally important consideration when examining fatigue status. St Clair Gibson et al. (2018) describe this wholistic view as an 'Integrative Central Governor' concept, whereby a barrage of sensory feedback occurs as a result of the alterations of the psychobiological state of an individual.

The ability to normalize or even optimise these markers, and hence, advance the status of recovery, means that athletes are able to maximise their subsequent performance or training effectiveness. As such, research surrounding recovery modalities has taken a major focus in the past twenty-five years, particularly the use of cold therapies (e.g., extreme cold air, and cold-water immersion (CWI)). The potential benefits of influencing the acute stress responses following exercise and enhancing recovery is an obvious attractive proposal to athletes and coaches.

Whole-body cryotherapy (WBC) is a therapeutic modality that uses short duration exposures of up to three minutes inside either a fully contained or head-exposed chamber in extreme cold air temperatures as low as -140 °C (Banfi et al., 2010). The reported benefits since its initial therapeutic use in the 1980s are varied from reduced pain levels experienced by patients with arthritic conditions, enhanced tissue regenerative processes, and reductions in sensations of fatigue (Taletavičienė et al., 2012). Mechanisms which may explain responses to WBC have been evidenced whereby stimulation of the parasympathetic nervous system appears to be induced by WBC (Hausswirth et al., 2011; Zalewski et al., 2014; Louis et al., 2015).

The use of WBC in Sports Medicine has increased in its popularity amongst sports teams and individual athletes, however, there remains limited evidence regarding its mechanistic effects upon aforementioned aspects of recovery (Rose et al., 2017). The body of literature surrounding the use of WBC in sports medicine has a history of approximately fifteen years, dating to the early-mid 2000s, with investigators frequently observing its effects in offsetting fatigue during a training period in the applied field (Wozniak et al., 2007a; Ziemann et al., 2012; Wozniak et al., 2013; Grasso et al., 2014; Schaal et al., 2015).

Hohenhauer et al. (2015) published a meta-analysis on the effects of post-exercise cooling which combined the data from studies involving cold water immersion (CWI), localized cold pack application, and WBC. Despite the authors establishing favourable effects of cooling modalities versus passive recovery, more beneficial outcomes in studies using CWI were shown. However, the authors conceded that the effects of WBC were largely unknown at this point in time given that only two studies involving WBC were analyzed in the review (Costello et al., 2012; Hausswirth et al., 2011).

Whilst some controlled laboratory studies have been conducted, they are limited in number, and so a systematic review by Rose et al. (2017) included both laboratory (N = 10) and applied training (N = 6) studies in assessing the effects of WBC. The key markers for observing effectiveness used in this review were indicators of pain, muscle damage, inflammation and performance. As a meta-analysis was not conducted in this study and only vote-counting to represent WBC modality effectiveness was employed, which naturally hinders accurate inference in understanding the effects of WBC (Borenstein et al., 2009). Further, studies using a partial body cryotherapy (PBC) modality, where the head is free from direct extreme cold exposure were pooled with WBC studies, and therefore the effects of the varied approach in delivering an extreme cold stimulus is unknown. Most studies included in the review by Rose et al. utilized WBC as an addition to a training period, and as such, the effectiveness of WBC used to influence a defined post-activity recovery period also remains unknown. Further laboratory and applied field studies have been published since 2016 (the limit of the inclusion period for the aforementioned review) that involve a wider perspective of the influence of WBC upon the mechanics of recovery (Russell et al., 2017; Douzi et al., 2018; Hohenhauer et al., 2018; Wilson et al., 2018; Douzi et al., 2019; Wilson et al., 2019). Therefore, an updated and more focused review of the effects of WBC upon post-exercise recovery is warranted.

The aim of this systematic review was to analyse the effects of extreme cold modalities (WBC and PBC) upon recovery following exercise or physical activities in trained populations. Comparisons with a control trial or alternative treatment were sought, where the markers assessed included biochemical, endocrine, inflammatory, and perceived ratings of fatigue.

Methods

Protocol

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The PRISMA checklist in Table 3.8 (Study Appendix) shows where items of information are present within this review chapter of the thesis.

Eligibility criteria and study selection

The use of a PICO (population, intervention, comparison, outcomes) model was employed to assess the final eligibility of an article. Eligible publications following this initial screening were examined for inclusion criteria covering the following:

- Studies published in English.
- Inclusion of athletes, trained participants or uninjured healthy populations, not restricting by gender.
- Inclusion of a defined whole-body or partial body cryotherapy intervention used post-activity without the influence of continual training or continual competitive activity.
- A treatment temperature range which reflects the scope of commercially available cryotherapy chambers (-85°C to -190°C)
- Use of a comparative trial, whether a control/passive or alternative recovery method.
- Inclusion of an observed recovery period (minimum of 12 h) from a defined exercise task that includes a measurable identifiers of fatigue status or influence upon recovery.

In order to assess relevance to the review, where the information in a study title or abstract was lacking the complete paper was obtained and read. Reviews, editorials, and conference abstracts were excluded. WBC or PBC was defined as the use of a purpose-built chamber in which its internal temperature in reaching and maintaining between -85°C and -190°C using forced convection or nitrogen gas could be controlled, and where participants were able to stand freely within the chamber. Papers were also accepted whereby the whole-body was enclosed within the treatment chamber, but with the head was exposed outside of the chamber (PBC) so long as the criteria above was achieved. This inclusion was justified since the commercially used modality of PBC is interchangeably referred to as WBC or PBC. Furthermore, it may be the case that due to the inclusion or exclusion of head exposure during WBC and PBC respectively, the effects may be different as proposed by recent research (Fonda et al., 2014; Louis et al., 2015). The exposure time for the participants in both WBC and PBC studies was aligned with chamber manufacturers'

guidance of between 2 and 4 minutes of treatment within the coldest region of the chamber, however, no restrictions were made in regard to treatment or dosage frequency. Articles were to have a clear exercise task or competitive activity which was sufficient to induce a state of fatigue or tissue damage. Publication language restriction has been shown to not affect bias in systematic reviews in conventional medicine studies (Morrison et al., 2012).

Literature search strategy and information sources

A systematic search of published peer-reviewed literature followed a process of identification, screening, eligibility, and inclusion using the standard PRISMA flowchart (Figure 3.1). This followed a strategy based upon two recent reviews in relation to this field (Rose et al., 2017; Hohenauer et al., 2015). The literature search was carried out between January and March 2020 using the following databases: SportDISCUS, Science Direct, ProQuest Central, CINAHL, Scopus, PubMed, and Google Scholar. The primary keyword search terms (using Boolean logic, AND / OR) included: 'whole-body cryotherapy' OR 'whole-body cryostimulation' OR 'partial body cryotherapy' in the title, abstract or article key words, AND secondary keyword search terms 'recovery' OR 'exercise' OR 'damage' OR 'athlete' found in the abstract.

Data extraction

The thesis author (AN) performed the initial data extraction, and a data check was performed by research colleagues (BE/CR). The following information was extracted from each study:

- Study authors and date
- Number and characteristics of the participants
- The parameters of the exercise / physical activity protocol
- The time points of measurement
- The treatment protocol details (WBC/PBC / comparison / control)
- The timing of WBC/PBC treatment relative to the exercise protocol
- Markers relevant to this review (physiological, biochemical, endocrine, inflammatory or perceptual)

- Statistics extracted, where reported, included mean ± 1SD, confidence intervals or percentage change from baseline measures.

Data analysis

Where data were not available, estimation from graphs was employed (Hohenauer et al., 2015). All data were then converted to percentage change from a pre-fatigued baseline (0 %) and expressed as mean percentage change and standard error where data are presented. It was decided that a meta-analysis for any outcome measure was unattainable due to the insufficient level of individual study data available to accurately compute collective effect sizes. There was clear methodological heterogeneity across the exercise protocols to instigate a consistent level of fatigue, and also, intervention protocols of WBC/PBC in terms of timing, treatment frequency and dose frequency further preventing a meaningful meta-analysis in this instance (Borenstein et al., 2009). Therefore, where common measurement time points were identified, pooled data were summarized descriptively using mean ± standard error of percentage change from baseline measures (Figures 3.2 – 3.4).

Quality and risk of bias assessment

The Downs and Black (1998) checklist is a comprehensive critical appraisal tool used in health care studies to assess quantitative studies for methodological rigor. This was employed in order to analyse study quality (Table 3.5). The Cochrane Risk of Bias tool was used to assess the level of potential bias within the reviewed studies. These assessments were conducted by the thesis author (AN) and used to inform the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) analysis to assess strength ranking of the overall body of literature (Table 3.7).

Results

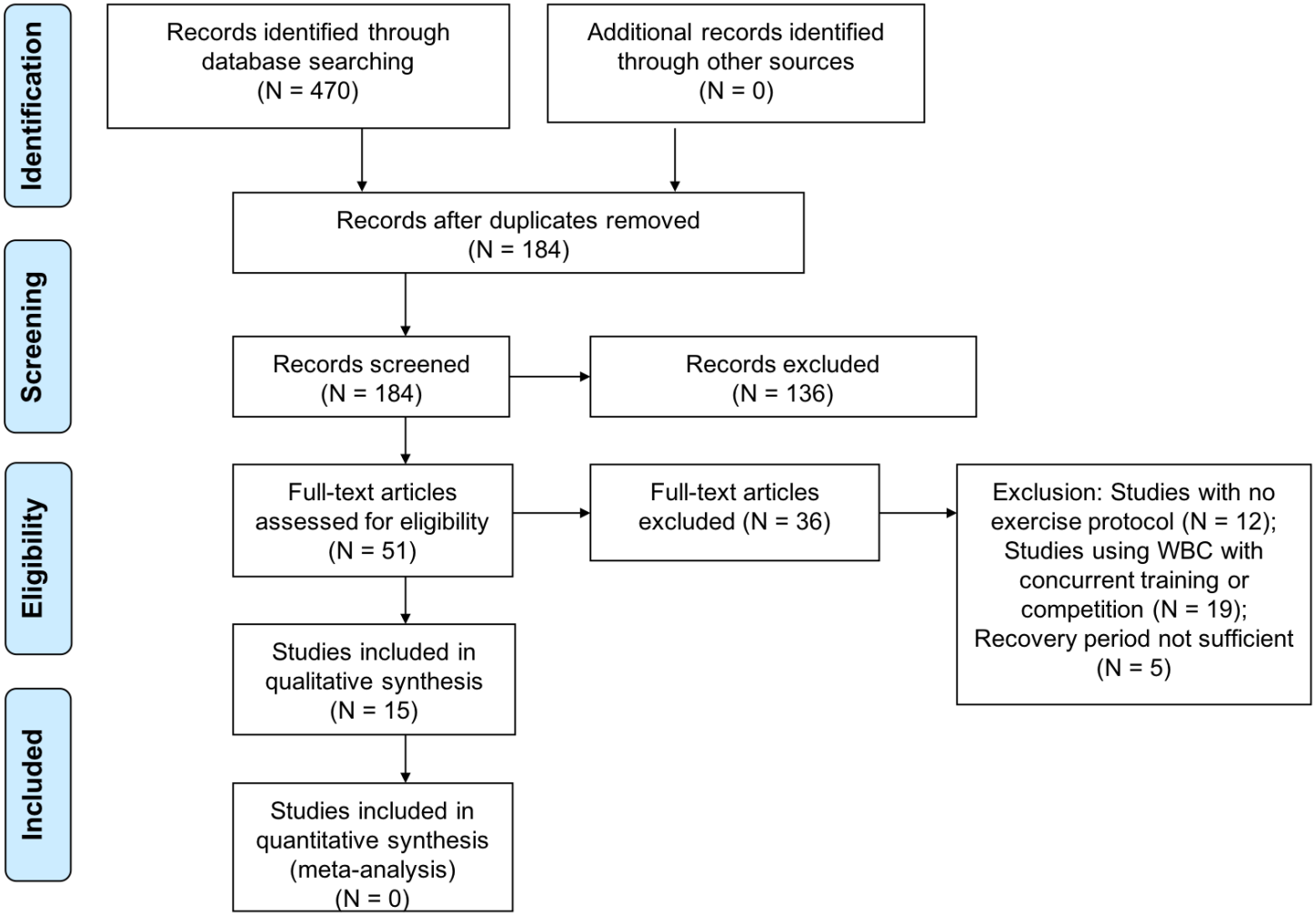

Figure 3.1. PRISMA flowchart used to screen and identify eligible literature for this review.

Search results

A total of 470 articles were captured using the primary and secondary search terms. Following the removal of 286 duplicate papers during search findings, the screening process on the basis of title and abstract excluded irrelevant subject areas, literature representing reviews, conference proceedings, abstract-only reports, unavailable abstracts, articles not written in English, experiments with non-human participants and articles not published in peer reviewed literature. Therefore, acceptable inclusion for screening purposes included relevance to the nature of the review title particularly in context of the secondary search terms used, random controlled trials (RCTs), uncontrolled trials, parallel, and crossover designs. Fifty-one full text articles met the inclusion criteria for further eligibility screening. The inclusion of none-randomised study designs (e.g., in environments where participant and/or trial randomization may be restricted) was justified given that a balance of internal validity and external relevance should be considered since extreme cold therapy is a growing clinical and field-based modality; solely focusing on RCTs would narrow the view of the effectiveness of WBC to controlled environments.

The articles excluded during eligibility screening (N = 36, see Figure 3.1) were on the basis that (i) the application of WBC/PBC was concurrent within training blocks, and therefore establishing specific recovery characteristics could not be done (N = 19); (ii) the post WBC/PBC period was not sufficient enough (<12 h) to observe adequate recovery characteristics (N = 5); (iii) no exercise or fatigue protocol was included in the study (N = 12).

Study characteristics

A total of fifteen papers met the screening eligibility and inclusion criteria for analysis. Ten studies used WBC as the intervention modality (Table 3.1), whilst five studies used PBC (Table 3.3). Five WBC studies observed significant benefits to a range of recovery markers, three studies showed indications of improved measures, and two studies observed inconclusive or no benefits of WBC (Table 3.2). With regards to PBC recovery intervention, three studies demonstrated any clear benefits to study outcomes measures, with one study showing inconclusive benefits in the markers

observed, and one study with unfavourable outcomes in comparison to CWI (Table 3.4).

Study quality and risk of bias

The Downs and Black (1998) analysis for study bias as shown in Table 3.5 gives a clear view that common methodological challenges remain consistent within this field of study. The key factor is blinding of the participants and/or assessors to extreme cold exposure (criteria 14 and 15), population representation (criterion 11) accounting for this and other confounding variable(s) (criterion 25). From this analysis, a risk of bias summary was made in order to inform the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) analysis (Figure 3.6, Table 3.6).

Participants

All studies involved a total of 244 participants, and were at least considered moderately physically active, typically defined as exercising at least three times per week. Participants across all studies were male, with the exception of one study which involved four female participants within its mixed sample (Costello et al., 2012). Six studies used a total of 87 male participants who considered highly trained or athletes (Hausswirth et al., 2011; Pournot et al., 2011; Russell et al., 2017; Wilson et al., 2018; Bieuzen et al., 2019; Douzi et al., 2019; Kruger et al., 2019).

Exercise protocols

Exercise protocols were varied across the studies. The six studies involved deliberate eccentric muscle contraction regimes, either though step ups/downs, drop jumping, or isolated leg curl/extension machinery (Costello et al., 2012; Fonda & Sarabon, 2013; Ziemann et al., 2014; Ferreira-Junior et al., 2015; Abaïdia et al., 2017; Hohenauer et al., 2018). One study followed a concentric-eccentric heavy training session of four weightlifting techniques (Wilson et al., 2019). Three studies reported data from a common source which used a simulated trial run on a treadmill (Hausswirth et al., 2011; Pounot et al., 2011; Bieuzen et al., 2019), and one study used an outdoor marathon distance trial (Wilson et al., 2018). The remaining four studies employed an

approach using interval running sets, one a maximal sprint speed, one at 90% of $\dot{V}O_2$max and one at 85 % of maximal running speed (Russell et al., 2017; Douzi et al., 2018; Douzi et al., 2019; Kruger et al., 2019).

Study design

Nine studies involved crossover (with between one- and four-week wash-out periods), repeated measure designs with the remaining six studies using parallel group comparisons. Eight out of the nine crossover studies utilized a control trial involving passive recovery, and a single crossover study used comparative PBC and cold-water immersion interventions. In thirteen of the studies, cryotherapy treatment applied following fatigue protocols was carried out within one hour. Two studies applied WBC 24 h post-activity (Costello et al., 2012; Ziemann et al., 2014). Cryotherapy temperatures typically remained across a range of -110°C and -140° C. Two studies (Wilson et al., 2018; Wilson et al., 2019) used temperatures of -85° C, and one study (Douzi et al., 2018) used a nitrogen-cooled chamber whereby a concurrent wind (equivalent to -60 °C) with a temperature of -40 °C was added, reporting a 'temperature feel' effect of up to -160 °C. Only four studies stated a consistent time of day that applied to the timing of the fatigue activity and subsequent measurement timings (Ziemann et al., 2014; Hohenhauer et al., 2018; Douzi et al., 2018; Douzi et al., 2019). The remaining studies did not specify a consistent time of day protocol, but rather maintained consistent intra-participant measurement timings to baselines or fatigue protocols during their respective recovery periods.

Markers of recovery

Twelve studies (eight WBC, four PBC) utilized a functional marker during the recovery period. These ranged from maximal voluntary contractions using isokinetic/isometric dynamometry (eight studies), vertical jump derivatives (six studies), physiological performance tests (two studies), and two studies monitored physiological responses and/or physical movement during sleep (Douzi et al., 2018; Douzi et al., 2019). Seven studies (five WBC, two PBC) used creatine kinase concentration as a marker of muscle damage and ten studies (six WBC, four PBC) used psychological markers and

scales to assess a perceived recovery state. Eight WBC studies used indicators representing immunological, endocrinological or inflammatory responses; none of the PBC studies used this group of markers.

Recovery periods and points of effectiveness

Monitored recovery periods ranged from 12 to 120 h following the application of a fatigue protocol (Figure 3.2). Varied timing points of effectiveness were identified across those studies observing significant or indications of change following the application of WBC (N = 7) or PBC (N = 4).

Effectiveness of WBC

From the eight studies showing indications of benefits from the use of WBC, five of these studies observed statistically significant alterations in recovery markers over passive recovery and CWI trials, while the remaining three studies showed some benefit via some measures (Table 3.3), and no benefit via others in comparison to passive recovery procedures. One study (Kruger et al., 2019) observed no difference to passive recovery procedures when using WBC, whilst Costello et al. (2012) showed similar recovery patterns between CWI and WBC trials.

Trends in specific recovery markers are shown in Figures 3.3 – 3.6 where common timepoint values could be pooled to demonstrate changes from baseline levels during the recovery process. Identifiable differences between control and WBC trials were evidenced in favour of WBC trials for testosterone (< 24 h), CRP (48 – 72 h), IL-10 (< 24 h), s-ICAM-1 (< 24 h), MVIC (24 – 72 h), and perceived soreness (24 – 72 h). Markers of CK appear worse under WBC conditions (24 – 72 h) compared to passive recovery.

Effectiveness of PBC

Five studies using PBC post-exercise were analysed, with three of these studies demonstrating statistically significant benefits to outcome measures (muscle swelling,

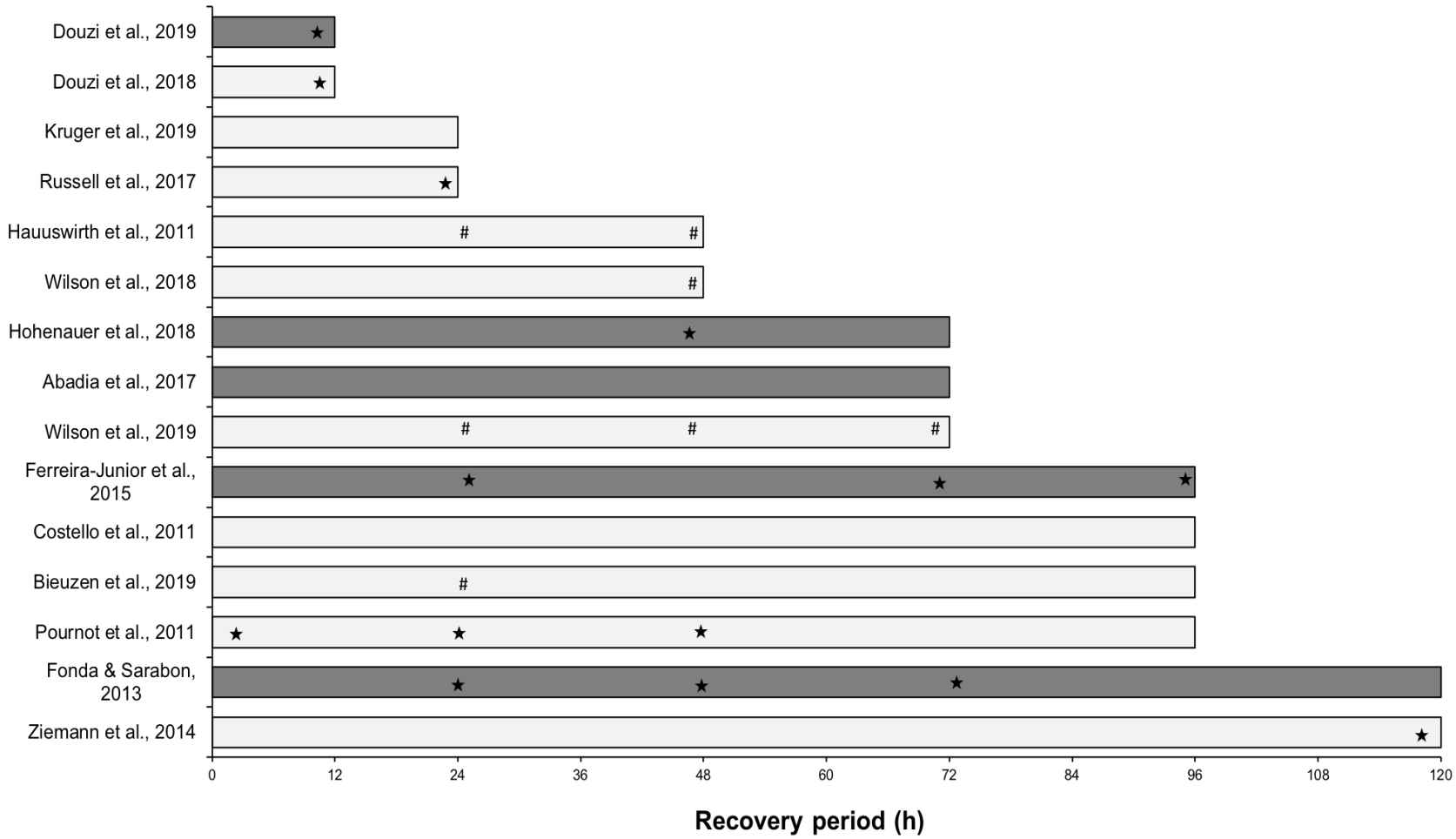

Figure 3.2. The recovery period involved in each study with point(s) of effectiveness detected in where applicable (clear bars denote WBC, dark bars denote PBC; ★ = time point of significant effectiveness; # = time point of potential effectiveness).

Table 3.1. Study design using WBC (NS = not stated).

Author(s), year	Participants	Exercise protocol	WBC treatment protocol	Treatment timing post-exercise (h)	Measurement time post-exercise (h)	Comparison	Time of day
Pournot et al., 2011	11 highly trained male runners	48 min treadmill simulated trail run (15 min downhill)	1 x WBC -110°C, 3 min	< 1, 24, 48, 72, 96	1, 24 ,48, 72, 96	Control crossover (4 wk wash out)	NS
Hauuswirth et al., 2011	9 highly trained male runners	48 min treadmill simulated trail run (15 min downhill)	1 x WBC -110°C, 3 min	< 1, 24, 48	1, 24 ,48	Control crossover (4 wk wash out)	NS
Costello et al., 2012	4 female, 14 male healthy university students	20 x 5 eccentric knee extensors @ 1.57 rad/s	1 x WBC -110°C, 3 min	24	24, 48, 72, 96	Control group (n=9)	NS
Ziemann et al., 2014	18 healthy college males	Step up/down (51 cm) 30 min 60% max aerobic power, 20 step/min	1 x WBC -110°C, 3 min, 09:30 and 15:00	24, 48, 72, 96, 120	120	Control group (n=9)	07:00 h - 08:30 h
Russell et al., 2017	14 professional academy male football players	15 x 30m sprint (10m deceleration), 60s rest	1 x WBC -135°C, 2 min	< 1	0, 2, 24	Control crossover (1 wk wash out)	NS
Douzi et al., 2018	22 moderately trained male runners, 1 h training x 3/ wk	55min track session, 25 min @65% max anaerobic sped, 3 x 7 min @85% max anaerobic speed, 2 min recoveries	1 x WBC -40°C + wind speed 2.3m/s (-60°C to -160°C equivalent), 3 min	< 1	12	Control crossover (2 wk wash out)	19:00 h
Wilson et al., 2018	31 trained endurance male runners	Outdoor marathon distance trial	2 x WBC -85°C, 3 min,	< 1	24, 48	A: CWI group 8°C, 10 min (n=11) ; B: Placebo group (n=10)	NS
Wilson et al., 2019	24 males (12-month strength training history)	4x6 back squat, 4x8 deadlift, hip thrust, split squat jump @ 80%1RM	2 x -85°C, 3 min	< 1	24, 48, 72	A: CWI 10°C, 10 min (n=8); B: Placebo pill (n=8)	NS
Bieuzen et al., 2019	11 highly trained male runners	48 min treadmill simulated trail run (15 min downhill)	1 x WBC -110°C, 3 min	< 1, 24, 48, 72, 96	1, 24 ,48, 72, 96	Control crossover (4 wk wash out)	NS
Kruger et al., 2019	11 endurance trained male runners	Ramp to exhaustion, 5 min rest, 5 x 5 min HI running @ 90% Velocity max, 60 min rest/(+WBC), ramp to exhaustion	1 x WBC -110°C, 3 min	< 1	1, 4, 24	Control crossover (1 wk wash out)	NS

Table 3.2. Study outcome measures and effectiveness using WBC.

Author(s), year	Functional	Endocr/ Inflam	Biochem.	Perceptual	Main findings
Pournot et al., 2011		TNF-a, IL-6, IL-10, IL-1ß, IL-1ra, CRP, leukocytes			WBC Positive ($P < 0.05$) over PAS for IL-1ß, IL-1ra (1 h and 24 h post), CRP (all time points),
Hauuswirth et al., 2011	MVIC		CK	Pain, tiredness, wellbeing 0/100 (VAS)	WBC ($P < 0.05$) recovered MVIC at 1 h, infrared at 24 h, PAS at > 48 h. Pain, tiredness lower at 1 h and 24 h for WBC; wellbeing return to baseline at 1 h for WBC.
Costello et al., 2012	5 x 6s cycling peak power output, MVIC			Soreness 1-10 (VAS)	WBC No significant different to control group
Ziemann et al., 2014	Step up/down 30 min, Respiratory exchange ratio	IL-6, IL-10, basophils	CK	Pain 0-10 (VAS)	WBC Positive over control group 24 h post-exercise after 10 x WBC treatments for CK, IL-1ß, IL-10 ($P < 0.05$)
Russell et al., 2017	CMJ, PPO	Cortisol, testosterone T/C ratio	CK, BLac	Perceived recovery 0-10, Soreness 0-7 (Likert)	WBC Positive over control trial for testosterone ($P < 0.05$)
Douzi et al., 2018	HRV R-R intervals during sleep, accelerometery for sleep movements			Fatigue, pain 0-10 (VAS), SSQQ	WBC Positive over control trial: significant ($P < 0.05$) HRV change in first 10 min SWS period, reduction in sleep movements and increased sleep efficiency; higher SSQQ score.
Wilson et al., 2018	IKD PT 60°/s; MVIC, RSI	IL-6, CRP, TNF-a	CK	Soreness 0-10 (Likert) DALDA	Effects are unclear; some indication for WBC benefits in perceptual responses at 24 and 48 h, and lower CRP at 24 and 48 h.
Wilson et al., 2019	RSI, MVIC, IKD PT 60°/s, CMJ Height, RFD	IL-6, CRP, TNF-a	CK	DALDA, Soreness 0-10 (Likert)	Effects are unclear; some indication for WBC benefits in perceptual responses at 24 h.
Bieuzen et al., 2019		sICAM-1			WBC inconclusive compared to PAS; indications for lower sICAM-1 at 24 h after WBC.
Kruger et al., 2019		IL-6, IL-10, sICAM-1, CRP, Cortisol, testosterone			WBC No significant difference to PAS

Table 3.3. Study design using PBC (NS = not stated).

Author(s), year	Participants	Exercise protocol	WBC/PBC treatment protocol	Treatment timing post-exercise (h)	Measurement points post-exercise (h)	Comparison	Time of day
Fonda & Sarabon, 2013	11 male healthy moderate physical activity	5 x 10 drop jumps, 5 x 10 bilateral prone leg curls (75% concentric 1RM), 10 eccentric curls @ 130% conc 1 RM	1 x PBC -140 < -195°C, 3 min	< 1	1, 24, 48, 72, 96, 120	Control crossover (10 wk washout)	NS
Ferreira-Junior et al., 2015	26 male university student, moderate physical activity 3 d/ wk	5 x 20 drop jumps 0.6m box, 2 min rests	1 x PBC -110°C, 3 mins	< 1	24, 48, 72, 96	Control group (n=13)	NS
Abadia et al., 2017	10 physically active males	5 x 15 IKD (60°/s) hamstring, 3 min recoveries	1 x PBC -110°C, 3 min	< 1	24, 48, 72	Crossover: CWI (10°C) 10 min (2 wk washout)	NS
Hohenauer et al., 2018	19 male, moderate physically active	5 x 20 drop jumps 0.6m box, 2 min rests	1 x PBC -135°C, 2 min	< 1	1, 24, 48, 72	CWI group (n = 9) 10 min 10°C	08:00 - 12:00 h
Douzi et al., 2019	9 male professional footballers	90 min football training session, (30 min ,15 s reps @95% maximal anaerobic speed, 20 min plyometrics)	1 x PBC, -180°C, [180 s] v [2 x 90 s] v [1 x 90 s] v control	< 1	18	Control crossover (1 wk washout)	12:00 h

Table 3.4. Study outcome measures and effectiveness using PBC.

Author(s), year	Functional	Biochemical	Perceptual	Main findings
Fonda & Sarabon, 2013	CMJ, MVIC, reactive torque development, squat jump	CK	Pain perception scale 0-10 VAS	Signfiicant positive influence of PBC over PAS: pain sensations < 72 h (P = 0.050 – 0.006), RFD 24 h (P = 0.012)
Ferreira-Junior et al., 2015	MVIC, thigh muscle thickness (swelling)		Soreness scale 0-100 mm VAS	PBC positive over PAS: muscle thickness l increased n PAS at 24 h (P < 0.05); MVIC recovered at 96 h, and soreness at 72 h in PBC (P < 0.05)
Abadia et al., 2017	MVIC, eccentric torque 60°/s, CMJ Height	CK	Soreness scale and perceived recovery Likert scale 0-10	PBC Less effective than CWI – moderate to likely effects in favour of CWI for soreness at 48 h, perceived recovery at 24 h, lower CK at 24 and 72 h, CMJ at 72 h,
Hohenauer et al., 2018	MVIC, CMJ Height, thigh muscle thickness (swelling)		Soreness scale 0-10 cm VAS	PBC inconclusive to CWI: muscle thickness increased in CWI at 48 h (P = 0.01), lower CMJ at 1 h in CWI (P = 0.01)
Douzi et al., 2019	Accelerometery for sleep movements		SSQQ	PBC 180 s positive over PAS and other PBC timings for sleep quality and reduced sleep movements (P < 0.01 – 0.05). Trend for sleep efficiency for 180 s (P < 0.09).

(Tables 3.1 – 3.4 abbreviations: CK = creatine kinase; CRP = C-reactive Protein; IL-6 = interleukin-6; IL-10 = interleukin-10; TNF-a = tumor necrosis factor a; WBC = whole-body cryotherapy; PBC = partial body cryotherapy; PAS = passive recovery; CWI = cold water immersion; MVIC = maximal voluntary isometric contraction; CMJ = countermovement jump; HRV = heart rate variability; SSQQ Spiegel Sleep Quality Questionnaire)

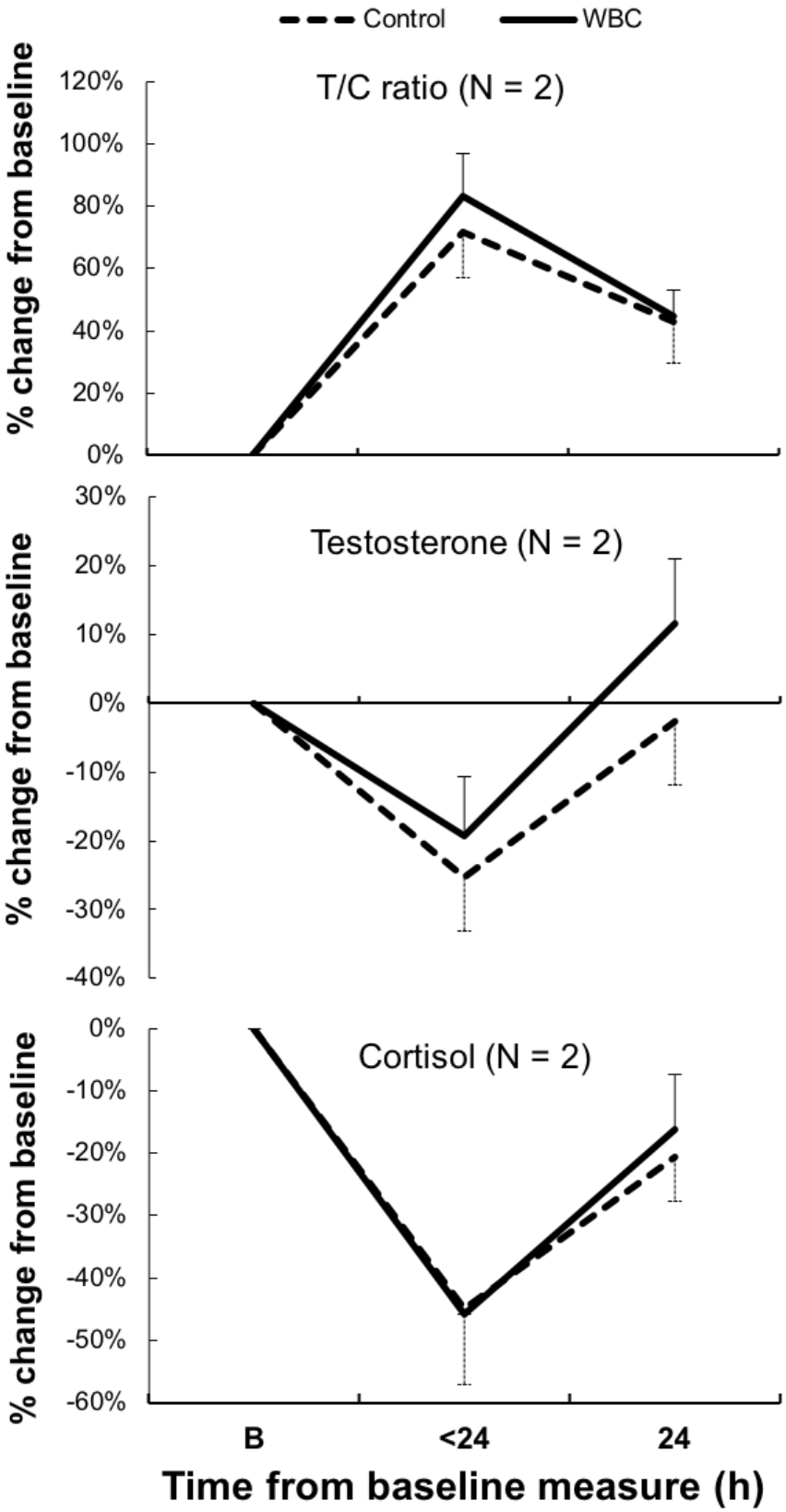

Figure 3.3. Mean and SE of endocrine measures up to 24 h post-physical activity comparing control and WBC only trials.

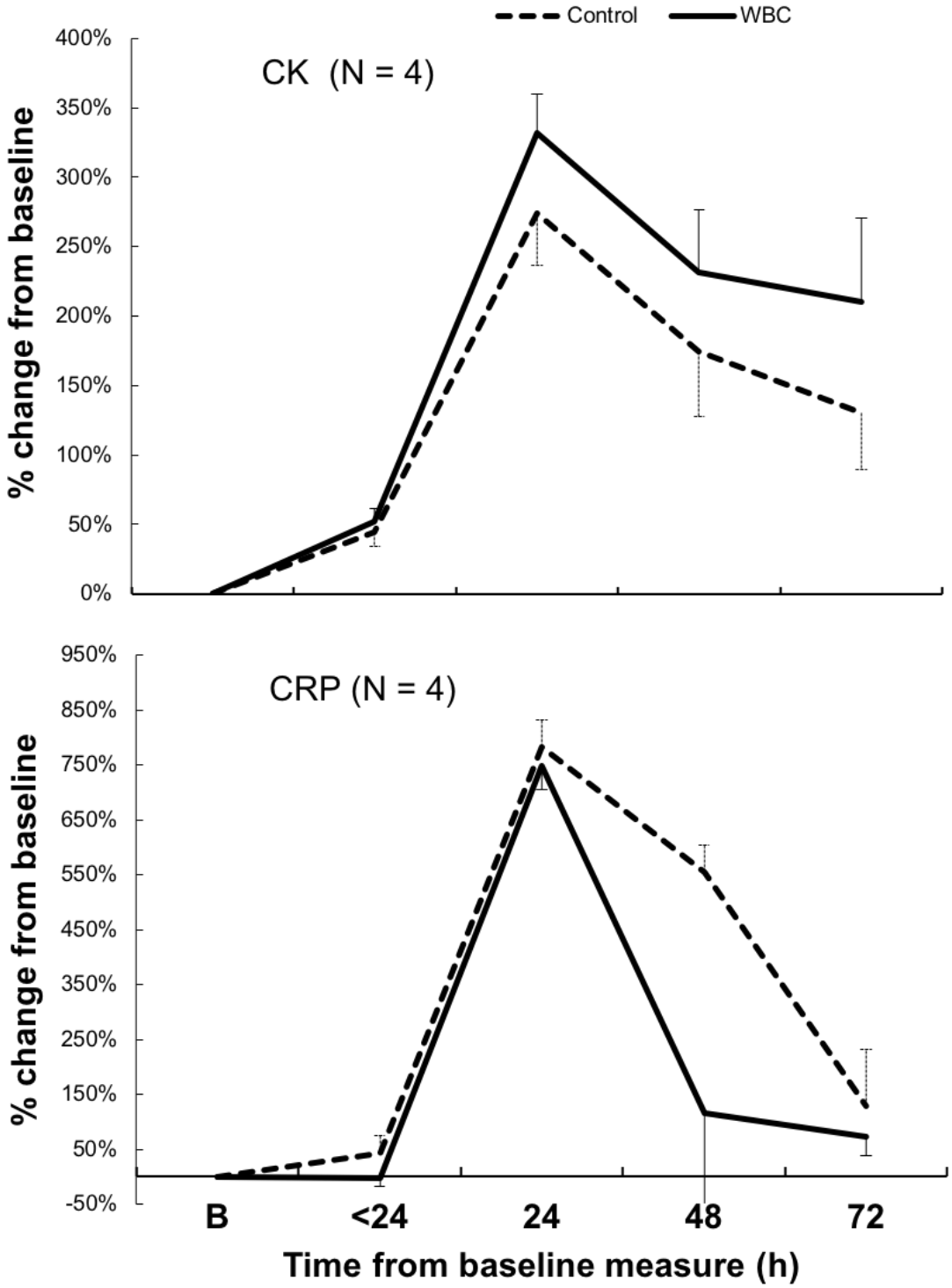

Figure 3.4. Mean and SE of CK (muscle damage) and CRP (inflammatory) measures during the first 72 h post-physical activity comparing control and WBC only trials.

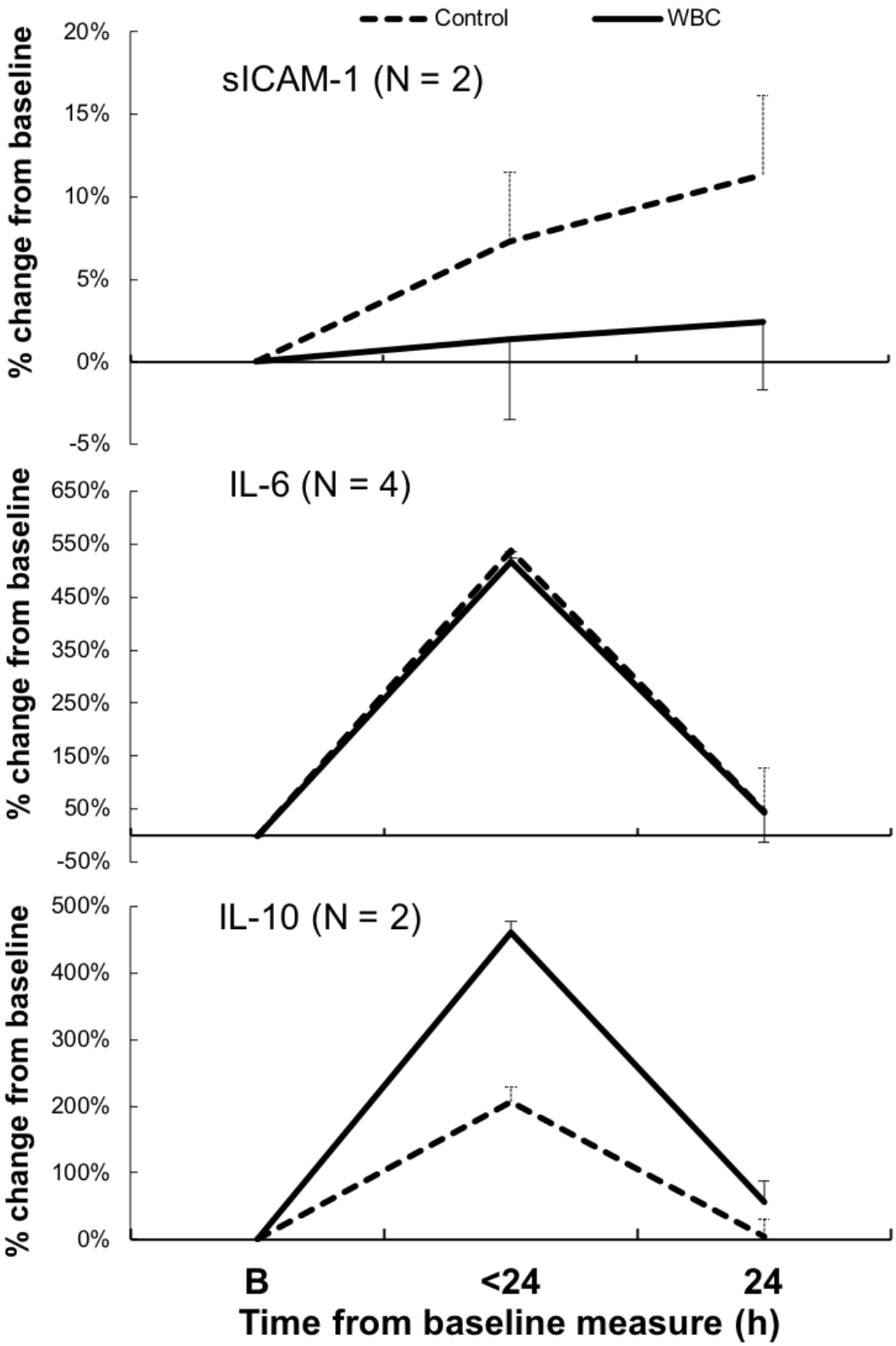

Figure 3.5. Mean and SE of inflammatory markers up to 72 h post-physical activity comparing control and WBC only trials.

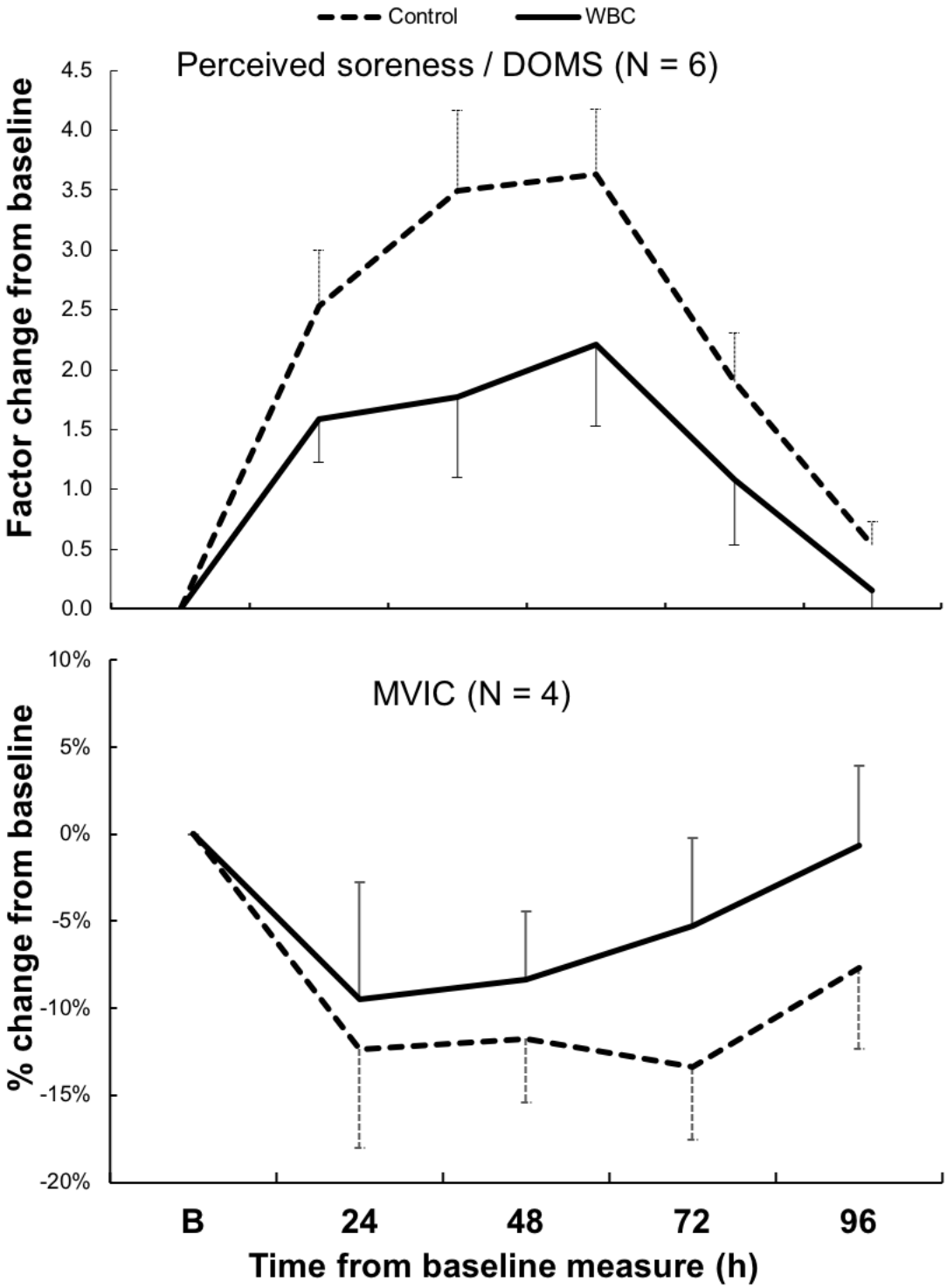

Figure 3.6. Mean and SE of perceived markers and MVIC measures up to 96 h post-physical activity comparing control and WBC only trials.

MVIC, and perceived soreness) in comparison to controlled conditions (Fonda and Sarabon, 2013; Ferreira-Junior et al., 2015; Douzi et al., 2019). The remaining two studies compared PBC and CWI only, and did not use a control group. One study was more favourable for CWI (Abaïdia et al., 2017), the other showing inconclusive data (Hohenauer et al., 2018).

Discussion

As an overall observation, despite half the number of studies utilizing PBC (N = 5) than those using WBC (N = 10), some benefits may exist through the utilization of either modality. Three out of five studies showed statistically significant benefits for PBC, and five out of ten for WBC, although positive trends not reaching significance were viewed in three further WBC studies. Only two studies had a particularly negative view of extreme cooling when compared to passive recovery (Wilson et al., 2018) or CWI (Abaïdia et al., 2017). The study by Wilson et al. (2018) utilized temperatures of -85 °C in a whole-body chamber following a simulated marathon run in endurance trained runners. Whilst a multifaceted state of fatigue was undoubtedly reached following marathon running of approximately 3.5 h, the observed 48-h recovery period was far too short to reflect meaningful change. Recently, Bernat-Adell et al. (2019) observed elevations of muscle damage and inflammatory markers between four- and six-days post-marathon racing in 86 runners, and therefore the study by Wilson et al. may well have missed the opportunity to observe post-48-h responses to WBC. Further the comparative temperature to all other studies (-85 °C compared to <-110 °C) the extreme cooling effect may not have had the same impact using despite two consecutive exposures. Wilson et al. (2019) showed similar inconclusive results using the same WBC protocol following resistance exercise which further supports the notion that less extreme temperatures than typical forms of cryotherapy delivery (i.e., warmer than -110 °C) may not elicit desired effects.

In support of negative effects of extreme cold, Abaïdia et al. (2017) did also observe lesser influences of PBC, but this was only in comparison to CWI, and since a control group was not included, any true negative effect of PBC against passive recovery therefore cannot be deduced in this case.

Whilst benefits of WBC/PBC have been observed in the majority of studies reviewed, the key consideration to note is the variability of positive/no change amongst concurrently measured variables. Table 3.2 (WBC) and 3.4 (PBC) demonstrate this variation across individual outcome measures, and grouping categories, for example, functional, inflammatory, biochemical, perceptual and endocrinological markers where some markers displayed statistically significant effects, and others did not.

Inflammatory and biochemical markers

Inflammatory markers were used only in the ten WBC studies, where only in 33 % of measured markers was any benefit shown (see Figures 3.3 and 3.4). The potential reasons for this are numerous. Firstly, the inflammatory response is dependent upon the level of stress and/or tissue damage caused, and so, some protocols may not have been sufficient to instigate similar responses (Mendham et al., 2011). Secondly, the concurrent responses of inflammatory and biochemical markers appear variable across the reviewed studies. For example, creatine kinase (CK) was not consistently affected by extreme cold, and so the singular use of CK should be approached with caution when employing this indicator as a recovery marker following extreme cold stimuli. Furthermore, it's representation as a marker of muscle damage as stated in many studies should also be carefully considered since changes in muscle function can be observed independently of CK change. As such, its expression as a marker of muscle cell membrane disruption should remain the focus in reflecting the status of cell integrity during recovery. Alternatively, cytokines responsive to inflammation such as IL-10, IL-6, s-ICAM1 and CRP demonstrated some degree of change. IL-6 does not appear to be impacted by WBC in its immediate response to physical activity, whereas IL-10, s-ICAM1 appear to alter concentrations within 24-h when WBC is used immediately post-activity. CRP also appears to be significantly reduced in concentration after 24-h post-activity under WBC conditions. Data from four studies were able to be pooled for CRP and IL-6, only two studies could be combined for IL-10 and s-ICAM. Other studies did also show some significant change in IL-1ß, and IL-1ra within 24 h (Pournot et al., 2011; Ziemann et al., 2014). IL-10 is a responsive anti-inflammatory cytokine, and so with the pooled observation which supports heightened IL-10 responses following WBC may be indicative of a protective effect of extreme cooling (Burmeister and Marriott, 2018). Reduced sICAM1 and CRP inflammatory

markers under WBC conditions also supports this view of a protective effect. Other studies showing no cytokine change include Wilson et al. (2018 and 2019) and Kruger et al. (2019). However, the WBC intervention (Wilson et al.), and fatigue protocol (Kruger et al.), within these studies are subtly different to studies showing positive effects.

In addition to the variable WBC temperatures used across protocols, the other key confounding issue is related to heterogeneity of the preceding task across studies. This variation across exercise protocols, opens larger potential for broad individual responses to physical tasks, since they are dependent upon the intensity of the damage stimulus, particularly, high intensity eccentric exercise (Bruunsgaard et al., 1997). The studies using inflammatory markers did use eccentric exercise as part of their protocols, however, only to either an intensity of 80 %RM or moderate intensity running/stepping activities using participant's own body weight. It may be that maximal efforts and those generated during highly stressful competitive situations align with a more consistent systemic release of cytokines when creating a more reliable view of a fatigue state. Vargas & Marino (2014) discuss a 'danger signal' reflex involving cytokines and a neuroinflammatory afferent connection between the periphery and brain. The resultant vagal and sympathetic tone instigated through this feedback is therefore heightened with higher intensities and greater tissue and metabolic disruption. As such, the extreme cold stimulus may also only significantly interact with greater disruption of homeostasis. Studies reviewed that employed intensities of 85% or greater all displayed a positive effect of WBC and PBC on recovery markers. Costello et al. (2012) did also use very high isokinetic intensities in their fatigue protocol however, the application of WBC was delayed by 24-h after activity. With this point in mind, the timing of WBC may also be an influential factor of effectiveness which requires further investigation.

Functional markers

Functional measures do also show variation in response to WBC or PBC, particularly neuromuscular outputs such as maximal isometric, isokinetic or vertical jumping outputs, where outcomes either show 'no change' or 'positive change'. Given that maximal outputs require a high central drive, the variation in exercise protocols used

across the studies may not have fully stressed the components of central neuromuscular co-ordination. As such, maximal measures may not accurately reflect the levels of fatigue created in each of the studies (Marshall et al., 2015). Figure 3.5 indicates distinctly improved recovery of MVIC from 72 h onwards when influenced by WBC. However, since three out of the four studies included in this particular pooled analysis did not show statistical differences across trials, there may be a compounding effect of data pooling which may demonstrate imprecision from this visual inference. As a result, a maximal output functional measure may not be the most optimal marker to compare across studies, and so the extent of fatigue should always be considered when interpreting the rate of recovery from activity.

Endocrine markers

Endocrine markers are under investigated in the literature, appearing in only two manuscripts. Russell et al. (2017) observed early indications of increased testosterone concentrations 24-h after exposure to WBC stimulation following maximal repeated sprint activities in academy level footballers when compared to a control trial. Kruger et al. (2019) did not observe a change in testosterone levels; however, the exercise trial did use repeated high intensity running at 90 % of maximal velocity, a 60-minute rest period, subsequent WBC intervention and then a post-WBC ramp to exhaustion. Both investigations did not collect data beyond 24 h, and so, there appears to be indications that uninterrupted recovery is required once WBC has been applied at least in the short term (e.g., <24 h) to gain a positive effect. Neither study observed significant changes in cortisol responses, although Kruger et al. did show indications of lowered mean cortisol concentrations in the WBC trial after 1 h and 4 h following the ramp to exhaustion, however this was not statistically significant. Whether or not a subsequent bout of exhausting exercise after a rest (once cortisol levels have elevated once already) is the driver to a secondary lowered cortisol level, or the cold stimulus has a potential effect remains unknown. Other studies have shown recovery of cortisol concentrations of anywhere between 1 h to 48 h following exercise (Lac & Berthon, 2000; Cunniffe et al., 2010; McLellan, 2011b). Again, a dose response is indicated by previous research in terms of participant training status, whereby more trained individuals typically have a higher intensity threshold necessary to provoke an increase in cortisol levels, particularly above 80 % of maximal intensity (Hill et al.,

2008). Since this was reached in both studies, specificity and task familiarity should be considered, which in these studies, the physical tasks were controlled and defined. In uncontrolled competitive environments, the recovery is likely to be extended when multiple components of fatigue are instigated, for example, following competitive rugby matches, Cunniffe et al. (2010) and McLellan et al. (2011b) both observed extended time frames of cortisol normalization of up to 14 h and 48 h, respectively.

Finally, when considering hormonal analysis, it is important that the time of day of sampling is reported due to circadian variations (Haupt et al., 2021). However, sample collection times are not always reported in manuscripts, and so this limits the between-study comparison of responses. Given the lack of evidence in this area within laboratory-controlled and competitive environments, hormonal responses to WBC certainly warrants further investigation.

Subjective markers

The outcome measures that displayed the most consistent benefits to WBC across studies are those of a psychological or perceived nature. Soreness ratings (Figure 3.6) was pooled in six studies and showed a marked reduction in ratings under WBC conditions compared to control trials. The reasons for this trend, at this stage are unknown, since the benefits would appear symptomatic rather than based on functional, or inflammatory, therefore a basal level function may be the driver for enhanced feeling of recovery especially beyond 24 h (see Figure 3.6). However, there is growing evidence supporting parasympathetic regulation following WBC, above that observed with PBC which may be the underlying driver behind homeostatic mechanisms and resultant central modulation to influence psychobiology. In response to a single exposure and series of daily WBC *vs* PBC exposures compared to baseline control measures, skin temperature, core temperature, resting heart rate, heart rate variability (HRV), and blood pressure received significantly greater benefits from WBC than PBC (Hausswirth et al., 2011; Zalewski et al., 2014; Louis et al., 2015). Centralisation of blood flow, resultant increases in diastolic blood pressure, and increased HRV following WBC (and not PBC) are indications of sympathetic attenuation, and the inclusion of the head exposure to extreme cold may contribute to

the global vasoconstriction mechanism *via* cold trigemino-cardiac reflex receptors located in the face, which may augment vagal output to the heart (Louis et al., 2015).

Added to this, the two studies reviewed in this paper observing sleep quality (Douzi et al., 2018; Douzi et al., 2019) both showed clear indications of improved sleep characteristics through objective (both studies), and subjective (WBC only – Douzi et al., 2018) markers. Movement during sleep was considered a 'functional' objective marker in this review, however, is worth noting in light of the potential basal level effects as described above. Sleep movement analysis was conducted via accelerometry monitoring during the sleep period. Significant findings ($P < 0.05$ and $P < 0.01$, respectively) were noted for reduced movement during sleep following high intensity (>85% of maximal anaerobic speed) exercise bouts over 55 – 90 minutes. A greater effect alongside other markers was evident when WBC was used in the evening (Douzi et al., 2018), compared to PBC in the afternoon (Douzi et al., 2019). Given that professional team athletes were monitored in the 2019 study, it seems viable that the time of day of activity and application of extreme cold may have an increased influence upon a post-activity sleep period. This certainly warrants further investigation as to the perceived benefits of the use of extreme cooling upon sleep, following afternoon or evening activity where recovery is vital in the live sport environment.

Summary

The central responses to extreme cold stimuli, and furthermore, inclusive of head exposure appear to hold stronger support for the symptomatic observations shown during post-exercise recovery. Neuromuscular (MVIC) and CK marker responses appear highly variable across studies and therefore should be interpreted in context of other measured parameters within the monitoring of recovery. Inflammatory markers may only change in the short term (< 24 h) and so the opportunity to monitor this closely may be challenging. There is potential for further investigation of endocrine markers given the very low number of studies observing changes post physical activity. The perceived benefits hold great potential for future explorative study, especially in regard to the timing of WBC and associated activity. The beliefs held by the users of WBC or PBC should be important considerations when, ultimately, the

sensation of ‘full recovery’ is one which incorporates an influx of afferent feedback from all systems of the body (St Clair Gibson et al., 2018). Studies have yet to investigate beliefs pertaining WBC, and so would provide insight to the expectations or potential placebo effect of WBC.

Limitations

Despite the steadily growing number of studies over the past ten years which assess the effect of extreme cold on the recovery from a physical task, there will always remain an unavoidable limiting variable in all cryotherapy studies i.e., the inability to blind participants from WBC or PBC treatment (Table 3.5, criterion 15). This opens the possibility for a placebo effect in participants who are especially aware of the intended use of WBC.

The reported temperatures (-40 °C to -140°C) and duration of exposure (2-4 minutes) varied inside the cryotherapy unit across the studies investigated. This compromises the dose-response comparison between studies. Furthermore, the manner in which extreme cold temperatures are achieved is also variable. The whole-body chamber was different in three WBC studies whereby, the still-air temperature was dramatically lower than that typically recorded (-40 °C, Douzi et al., 2018, -85 °C Wilson et al., 2018 & 2019). In the study by Douzi and colleagues, a contrived wind chill effect was used to give a ‘feel’ of up to -160 °C. Otherwise, in the studies by Wilson et al. the recorded temperatures were reported to remain at -85 °C, again a considerable comparison when looking to form consensus views on WBC use.

As discussed above, exercise protocols differed considerably across studies, and therefore it is difficult to analyse the extent of post-activity fatigue and the dose effect of either WBC or PBC. As previously reviewed earlier in this thesis, the overall extent of fatigue caused by activity does dictate the level of homeostatic disruption which is required to be resolved (St Clair Gibson et al., 2018). Since the studies included in this review do not have consistency in the state of fatigue reached, the starting point of recovery, in real terms, therefore changes. Furthermore, when combined with the large variation in outcome measurements across the studies, the view of effectiveness becomes even more clouded. As shown in Tables 3.2 and 3.4, and Figures 3.3 – 3.6,

some outcome measures are sensitive to more consistent change than others (whether positive or negative). In particular, the popular measure of creatine kinase appears to be one of the least consistent measures, with positive, negative and unchanged across studies. Perhaps the most consistent measurement outcomes, particularly for WBC, are psychological/perceptual ratings, however, there are likely many contributing factors to the sensations of pain, function, and/or recovery and so the limitation in this case is highlighted by the fact that mechanistic effects would remain unidentified. Functional performance measures such as maximal isometric voluntary contractions or vertical jumping performance, similarly to CK in terms of variability and inconsistency, contain many physiological sub-variables which can potentially cause either (i) the real influential mechanism of change (if any) to be missed, or (ii) only part of a measure of 'performance' to be observed.

As with many studies in the Sport Science field, participant groups which do not represent an intended population, particularly elite or high-level sport, pose a limited and perhaps an unrealistic extrapolation of findings to these arenas. The Downs and Black Risk of Bias Analysis (Table 3.5, criterion 11) highlighted this point, and as such, the number of studies in the elite sport field involving WBC remains lacking. Only two studies in this review utilized sample groups representing high level sports participants, and so the findings of studies using non-elite samples should be utilised with caution when extrapolating findings (Russell et al., 2017; Douzi et al., 2019).

Methodological quality and control

All studies reached a quality assessment score of 67 – 81 % using the Downs and Black analysis (Table 3.5). Further scrutiny was completed in order to identify specific areas of bias in the reviewed studies (Table 3.6, Figure 3.5). This enabled a GRADE analysis (Table 3.7) to be performed incorporating the important limitations relevant to this current body of information. Overall, a GRADE analysis determined the current study quality to be of a low-moderate level for the reasons described below.

The issue of participant blinding is unavoidable and potentiates bias towards the effects of placebo. Some offset could be applied here in having experimental

assessors blinded to the intervention and analysis procedure, thereby removing any further external influence to the participants during the experimental procedures.

At present, the collective sample size across studies is small, and participant levels of physical training is wide ranging from elite athletes, moderately trained, to ‘physically active’ individuals. This immediately creates a large case for imprecision, since being able to analyse a more homogenous sample group would help to clarify effects which are specific or beneficial to certain sport environments such as rugby or football (i.e., contact, high intensity sports). As such, despite the present data being suggestive of positive influences of extreme cold for recovery, the context in which this is applied requires further research; it has already been established that trained, elite-level individuals respond to physical stress differently to lower trained populations (Cadore et al., 2008).

The GRADE analysis raised the issue of indirectness, which hinders the extent of external validity of this data set. A large variation in physical activity protocols creates varying levels of fatigue states from which one has to recover. Coupled with the fact that 98% of the current participant group are male further limits the ability to apply the findings to contexts in the competitive arena for specific recovery. As such, study findings are in danger of being generalized to uninvestigated groups or post-activity states.

From a positive perspective, all studies ensured that randomization and/or counterbalancing of trials and groups occurred, whilst only two studies lacked control groups on which to compare the null effects of extreme cooling (Abaïdia et al., 2017; Hohenauer et al., 2018). The use of CWI as a comparator is useful, however, studies should aim to maintain a controlled situation in order to make meaningful comparisons between modalities as in the cases of Wilson et al. (2018; 2019).

Table 3.5. Downs and Black Risk of Bias Analysis (Downs & Black, 1998). 1 = criteria met; 0 = criteria not met; 0* = unable to determine if criteria were met; n/a = not applicable; * = Reporting category includes items such as study aims, reported outcomes, participant characteristics, interventions, confounders, adverse events, participant loss, reporting of probability; § = External validity category includes items regarding study population; ß = Bias internal validity includes items such as blinding, data dredging, data follow up, statistical tests, study compliance; ‡ = Confounding internal validity includes items such as randomization, study power.

	Author(s), year	Reporting*										External Validity§			Internal Validity													Total	% Rating	Quality
															Bias ß						Confounding‡									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27			
WBC studies	Pournot et al. (2011)	1	1	1	1	2	1	1	1	1	0	0*	1	NA	0	0	1	1	1	1	1	1	0*	1	0*	0*	1	1	20	74	Moderate
	Hauuswirth et al. (2011)	1	1	1	1	2	1	1	1	1	0	0*	1	NA	0	0	1	1	1	1	1	1	0*	1	0*	0*	1	1	20	74	Moderate
	Costello et al. (2012)	1	1	1	1	1	1	1	1	1	1	0*	0	NA	0	1	1	1	1	1	1	1	1	1	1	1	1	1	23	85	High
	Ziemann et al. (2014)	1	1	1	1	2	1	1	1	1	1	0*	0	NA	0	0	1	1	1	1	1	1	1	1	0*	0*	1	1	21	78	High
	Russell et al. (2017)	1	1	1	1	1	1	1	1	1	1	0*	1	NA	0	0	1	1	1	1	1	1	1	1	1	0*	1	1	22	81	High
	Douzi et al. (2018)	1	1	1	1	1	1	1	1	1	0	0*	1	NA	0	0	1	1	1	1	1	1	0*	1	1	0*	1	1	20	74	Moderate
	Wilson et al. (2018)	1	1	1	1	2	1	1	1	1	0	0*	1	NA	0	0	1	1	1	1	1	1	1	1	0*	0*	1	1	21	78	High
	Wilson et al. (2019)	1	1	1	1	2	1	1	1	1	1	0*	1	NA	0	0	1	1	1	1	1	1	1	0	0*	0*	1	1	21	78	High
	Bieuzen et al. (2019)	1	1	1	1	2	1	1	1	1	0	0*	1	NA	0	0	1	1	1	1	1	1	0*	1	0*	0*	1	1	20	74	Moderate
	Kruger et al. (2019)	1	1	1	1	2	1	1	1	1	1	0*	1	NA	0	0	1	1	1	1	1	1	0*	1	1	0*	1	1	22	81	High
PBC studies	Fonda & Sarabon (2013)	1	1	1	1	1	1	1	1	1	1	0*	0	NA	0	0	1	1	1	1	1	1	0*	1	1	0*	1	1	20	74	Moderate
	Ferreira-Junior et al. (2015)	1	1	1	1	1	1	1	1	1	0	0*	0	NA	0	0	1	1	1	1	1	1	0*	1	1	0*	1	1	19	70	Moderate
	Abadia et al. (2017)	1	1	1	1	1	1	1	1	1	0	0*	0	NA	0	0	1	1	1	1	1	1	0*	1	1	0*	1	1	19	70	Moderate
	Hohenauer et al. (2018)	1	1	1	1	2	1	1	1	1	1	0*	0	NA	0	0	1	1	1	1	1	1	1	1	0*	0*	1	1	21	78	High
	Douzi et al. (2019)	1	1	1	1	1	1	1	1	1	0	0*	1	NA	0	0	1	1	1	1	1	1	1	1	0*	0*	1	1	20	74	Moderate

Table 3.6. Risk of bias chart for each included study.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) Participants	Blinding (performance bias and detection bias) Personnel	Blinding (performance bias and detection bias) Outcome assessor	Incomplete outcome data	Selective reporting
Pournot et al., 2011	?	×	×	×	×	✓	✓
Hauuswirth et al., 2011	?	×	×	×	×	✓	✓
Costello et al., 2011	✓	✓	×	✓	?	✓	✓
Ziemann et al., 2014	?	?	×	×	×	✓	✓
Russell et al., 2017	✓	×	×	×	×	✓	✓
Douzi et al., 2018	✓	?	×	×	×	✓	✓
Wilson et al., 2018	?	?	×	×	×	✓	✓
Wilson et al., 2019	?	?	×	×	×	✓	✓
Bieuzen et al., 2019	?	×	×	×	×	✓	✓
Kruger et al., 2019	✓	×	×	×	×	✓	✓
Fonda & Sarabon, 2013	✓	?	×	×	×	✓	✓
Ferreira-Junior et al., 2015	✓	?	×	×	×	✓	✓
Abadia et al., 2017	✓	×	×	×	×	✓	✓
Hohenauer et al., 2018	?	?	×	×	×	✓	✓
Douzi et al., 2019	?	?	×	×	?	✓	✓

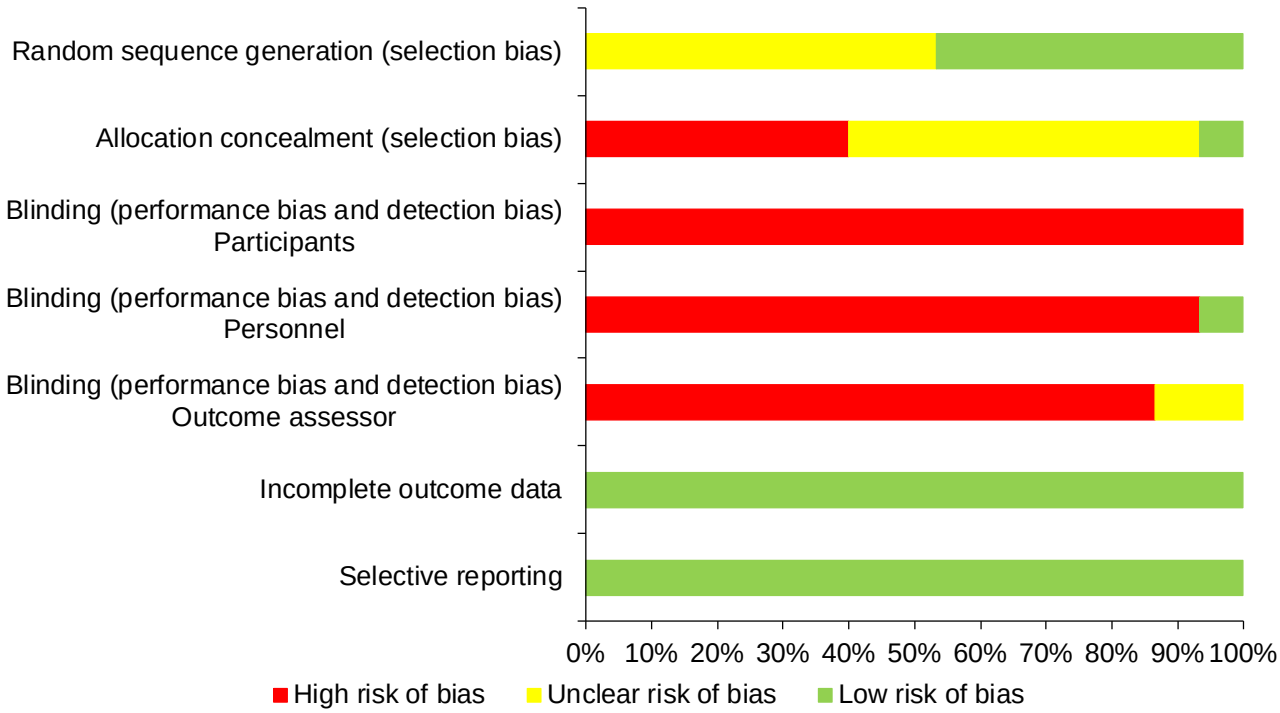

Figure 3.7. Summary of risk of bias assessment.

Table 3.7. Summary of GRADE analysis.

Grading of recommendations, assessment, development and evaluation (GRADE) Analysis		Judgement	
Risk of Bias	Lack of ability to include blinding is a major concern. This holds a serious threat to placebo-driven effects despite overall strong Downs and Black scores for methodological/reporting strength.	-2	Downgrade
Inconsistency	Effects are generally in favour of WBC or PBC for different outcome measures (overall lack of harm); Control poses no real benefit over cooling, whilst CWI may induce similar effects to WBC/PBC.		No change in grading
Indirectness	All except 1 study uses male participants; large variation in exercise protocol; extent of fatigue/exercise effects requires more standardisation. Some risk of bias to infer potential effects, or lack thereof given that no comparative standardisation of pre-cooling (fatigue) status is identifiable. Application to sporting populations is frequent in the studies, reducing heterogeneity and external validity towards use in males who are athletically trained status.	-1	Downgrade
Imprecision	Small sample sizes used throughout; total number of participants across all studies is 244. Some risk of bias to infer the potential effects. Some statistical quality is compromised in not providing exact P-values, and graphical estimates for some descriptive mean and SD data.	-2	Downgrade
Publication bias	No conflicts of interest reported, any study funding typically emerged from universities, WBC/PBC units were be provided by either commercial companies, clinical facilities or sport institutions. Some risk of reporting bias identified when given the use of commercial WBC/PBC facilities.	-1	Downgrade
Other factors	Majority are RCTs, with control (1 non-randomised CT, 2 with CWI-only comparisons)		No change in grading

Conclusions

This systematic review has highlighted that despite the growing number of studies using extreme cold methods as a stimulus for recovery from exercise, there remains difficulties in the interpretation of findings, this seems due to the inconsistency of methods used in generating a fatigue state. Therefore, at present, it remains difficult to clearly elucidate the real benefits to post-exercise recovery or mechanism of influence of WBC or PBC on an inflammatory, neuromuscular or biochemical level. Where improved objective markers of recovery were observed, methodological inconsistencies across studies prevent these mechanisms being demonstrated. Furthermore, this is notwithstanding a large potential for placebo-driven changes given the unavoidable bias of non-blinding or modality concealment to extreme cold exposure. The clearest conclusion being that there appears to be more of a symptomatic or perceptual benefit to recovery using WBC, lesser so in the case of PBC. The possible mechanisms may be due to centralized and basal level responses involving stimulation of parasympathetic responses, improved sleep benefits which may be the underlying driver for recovery from activity.

As such, there are many areas to explore which can help gain further insight as to the impact and effects of extreme cooling, and this thesis will look to explore the following key themes raised in this review:

1. The perceptions and symptoms of fatigue and recovery within a homogenous group of elite collision sport athletes.
2. The perceived benefits and current practice of extreme cooling in a homogenous group of athletes following fatiguing activity.
3. The hormonal responses to extreme cooling during contact sport activity across extended live sport time frames, including competition and training situations.
4. The impact which treatment dosage of extreme cooling has (i.e., repeat exposures) upon competitive high intensity, contact sport activity in order to substantiate an intensity effect of treatment.

Appendix. Table 3.8 Prisma Checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	77
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	TBC for publication
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	77 - 79
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	79 - 80
METHODS			
Protocol and registration	5	Review protocol exists, it can be accessed via email request from study author	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	81
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	82
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	82
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	81 - 82
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	82 - 83
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	81 - 82
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	83
Summary measures	13	State the principal summary measures – outcome measures relative to physiological, biochemical, endocrine, inflammatory, perceptual markers summarised as mean ± SD, confidence intervals or percentage change from baseline measures	82 -83, Tables 3.1-3.4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency – pooled data for mean ± SE of % change from baseline.	83
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	83
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	n/a
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	84 - 85
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	85–87; 89 - 91
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	105 - 107
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	93 - 96
Synthesis of results	21	Present results of each outcome measure summarised as pooled data for mean ± SE of % change from baseline	93 - 96
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	105 - 107
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	n/a
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	97 - 102
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	102 - 108
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	108 - 109
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	none

Chapter 4

The beliefs and perceptions of fatigue, recovery, and current practice of whole-body cryotherapy in elite contact sport.

Introduction

Optimal recovery from high intensity competitive sport such as rugby is critical due to the requirement to perform subsequent, optimal training or competition (Van Wyk & Lambert, 2009). Extensive states of fatigue are common following competition, and it is not uncommon for recovery to take up to five days in order to return to pre-match levels (Passelergue & Lac, 1999; McLean et al., 2010; McLellan et al., 2011a & b; Tavares et al., 2017). Regular training sessions between competitive fixtures frequently coincide with a status of fatigue, and therefore the recovery process becomes ever more critical in order to reduce injury risk and optimise performance. Additional strategies to benefit recovery are reported to be commonplace in recovery practices of athletes, however, a broad range are reported to be used (Venter, 2014; Van Wyk & Lambert, 2009).

Few studies have researched the perceptions and practices of recovery strategies either from the perspective of an athlete (Venter, 2014; Crowther et al., 2017; Murray et al., 2018), coach (Simjanovic et al., 2009), or practitioner (Van Wyk & Lambert, 2009). Recent findings indicate that perceptions of the benefits of a recovery strategy do not always align to scientific evidence (Crowther et al., 2017), and furthermore the practices implemented may be led by previous experiences of coaches, general observations, or instinct (Simjanovic et al., 2009). On the whole, only ratings for frequency, importance, and benefits of use have been reported in these studies. The reasoning or beliefs for using recovery modalities has been explored by one study only; this was in relation to post-activity stretching, cold water immersion, contrast water therapy, and active recovery (Crowther et al., 2017). The use of whole-body cryotherapy has not been explored in this context despite the increase in perceived popularity in elite level teams, and the public domain (Patel et al., 2019; https://cryoaction.com/saracens-cryoaction-cryotherapy-chamber/, 2017; https://fcbusiness.co.uk/news/fulham-launch-world-first-in-cryotherapy/, 2017; https://cryoaction.com/leicester-city-cryoaction-whole-body-cryotherapy-chamber/, 2015).

A current review in rugby union and rugby league also highlights an increased need to investigate the 'adoption' and 'implementation' of prevention strategies within club

practices, rather than the ‘effectiveness’ of strategies where research is typically focussed (Barden et al., 2021). The systematic review of this thesis highlighted that there is a gradually expanding number of studies which have investigated the widespread effects of WBC. However, little remains known about its applied effects in live competitive environments. As such, only 2 studies have utilised elite team sport athletes in analysing the post-activity recovery benefits of WBC, and no post-competition recovery studies have been performed with rugby players (Russell et al., 2017; Douzi et al., 2019).

At present, there are no studies which address the practices, beliefs and perceptions of WBC as a recovery tool following competitive sport, despite the contemporary anecdotal sources mentioned earlier. Despite a number of studies assessing subjective fatigue responses to rugby activity (Twist et al., 2012; Shearer et al., 2015), the perceptions of how recovery strategies relate to fatigue states and how this may be facilitated has not been explored. The alignment of beliefs and perceptions between athletes, coaches and practitioners regarding recovery may well provide more effective practice in a live setting.

Therefore, the aims of this study are to investigate the perceptions and beliefs of fatigue, recovery, and current practice of whole-body cryotherapy in elite contact sport from the perspectives of the athlete, and non-athlete (coach, medical and sport science/strength and conditioning) groups.

Method

Study design

This study was a questionnaire-based, cross sectional descriptive survey design and was made accessible electronically using a specific uniform resource locator (url).

Participants

Players, coaches, and practitioners (medical, sport science/strength and conditioning (SpSci/S&C)) of elite level rugby league (European Super League), rugby union

(English Championship) and football (English Premier League, Championship) were invited to participate in the study. The geographical reach of participating teams covered teams in England. From the total number of twelve teams contacted (17% of the available teams across the leagues stated - five rugby league, three rugby union, four football), 115 participant surveys were returned for pooled analysis and are presented in Table 4.1.

A similar approach towards gathering participants to Crowther et al. (2017) was used. Club personnel (typically the medical staff) were contacted *via* email or phone in order to establish initial consent to share the survey url amongst colleagues and athletes. Contact was made through personal connections linked to the author's professional network.

Information regarding the purpose and content of the survey, and how anonymised data were to be used were explained on the opening survey webpage. It was explained that participants consented to their responses being included in the study analysis by submitting the survey upon completion. Participants were aware that they could withdraw their data and close the survey webpage at any point before the survey was submitted. Ethical approval for the study was granted by the University of Bolton research ethics committee.

Survey details

A pilot version of the survey was shared amongst academics, practitioners, coaches and players who were familiar with a similar level of competition (n = 11). Individual feedback from each pilot participant was compiled in order to inform the refinement of ambiguous questions, and a revised version with adjusted questions was re-shared with the pilot group. The approximate time of completion was 15 minutes across the pilot group. No further rounds of pilot feedback were requested due to time constraints in completing the study.

The survey focussed on four main sections. The first section consisted of demographic information regarding age, sport, role within the sport, the typical number of training sessions and competitive matches which the participant is involved in per week. The

second section explored the participants' perceptions of fatigue and recovery following training and matches. This consisted of reasons for fatigue, symptoms of fatigue, timeframe of recovery, monitoring of recovery, and a time-of-day influence upon fatigue and recovery from training and matches. The third section focussed upon use of and beliefs in cold modalities. In the final section, the focus was drawn towards experiences, beliefs, perceptions and practice (application parameters such as timing and dosage) of WBC following training and matches. Questions in this section were made optional depending upon whether the participant had experienced WBC or not (Q20). As such, instructions were provided following this section's initial question to guide the subsequent responses relating to WBC. The full survey can be found in the thesis appendices.

Questions were designed to incorporate (i) closed choice responses, (ii) Likert scale ratings, and (iii) open responses for a given closed choice. For some closed choice questions, multiple options were able to be selected concurrently.

Data analysis

A combination of quantitative and qualitative analyses was conducted. The quantitative data analysis was completed using Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL), IBM version 26. Data were presented as mean ± standard deviations, raw numbers of respondents (n), or proportions (%) of responses. Non-parametric statistical tests were utilised to establish differences between data gathered across respondent role (e.g., athletes *vs* non-athletes or coaches or practitioners), training and matches. Mann-Whitney and Kruskal Wallis Tests were used to compare between-participant frequencies and Wilcoxon Signed Rank Tests were used to compare within-participant frequencies. When the SPSS output demonstrated significance levels of $P = 0.000$, these were corrected to $P < 0.0005$ (Kinnear & Gray, 2006). A statistical trend was identified with $0.1 > P > 0.05$.

For qualitative analyses, open question responses were grouped into common themes and quoting text directly as specific examples (Crowther et al., 2017). The identification of themes was completed by the research author (AN).

Data was presented to represent the following areas:

- Demographic data
- Perceptions of fatigue and recovery
- Experiences and usefulness of monitoring fatigue
- Experiences and beliefs of cold therapy modalities
- Experiences and beliefs of WBC users
- Beliefs of non-frequent / non-users of WBC

Results

All participants (n = 115, age 28.1 ± 7.3 years) completed the first three sections of the survey. In section four, question 21 regarding WBC experiences was fully completed by 67 respondents and question 22 regarding intended use of WBC by 86 respondents. All respondents were involved in between 4 and >10 training sessions and 1-3 matches per week either as an athlete (age 25.2 ± 4.6 years or a non-athlete (coach, medical, or sport science/S&C practitioner, age 34.1 ± 8.1 years).

Demographic data (n = 115)

The demographic of the respondents is displayed in Table 4.1. The majority of respondents either participated or worked in Rugby League (73 %).

Perceptions of fatigue and recovery (n = 115)

The perception of fatigue usually occurring post-activity declined in training situations (67 % post-training *vs* 97 % post-match, see Figure 4.1). This appears to be accounted for by lower perceived occurrence of ‘damage to the body’ and ‘mental tiredness’ across all participant groups (Table 4.2). ‘Physical tiredness’ was reported consistently as the main reason for fatigue following match and training. A Kruskal-Wallis Test

revealed no difference between all respondent groups in perceived recovery time from matches before higher intensity activity can/should be applied ($\chi^2(3) = 2.512$, P = 0.473). On average, this timeframe was judged to be 3.0 ± 0.7 days.

Table 4.1. Demographic of the survey respondents (n, %).

Sport	Athlete	Coach	Medical practitioner	Sport Science / S&C	Total
Rugby League	66 (57.4)	3 (2.6)	14 (12.2)	1 (0.9)	84 (73)
Rugby Union	11 (9.6)	0 (0)	3 (2.6)	1 (0.9)	16 (14)
Football	1 (0.9)	1 (0.9)	9 (7.8)	5 (4.3)	15 (13)
Total	78 (68)	4 (3)	26 (23)	7 (6)	115 (100)

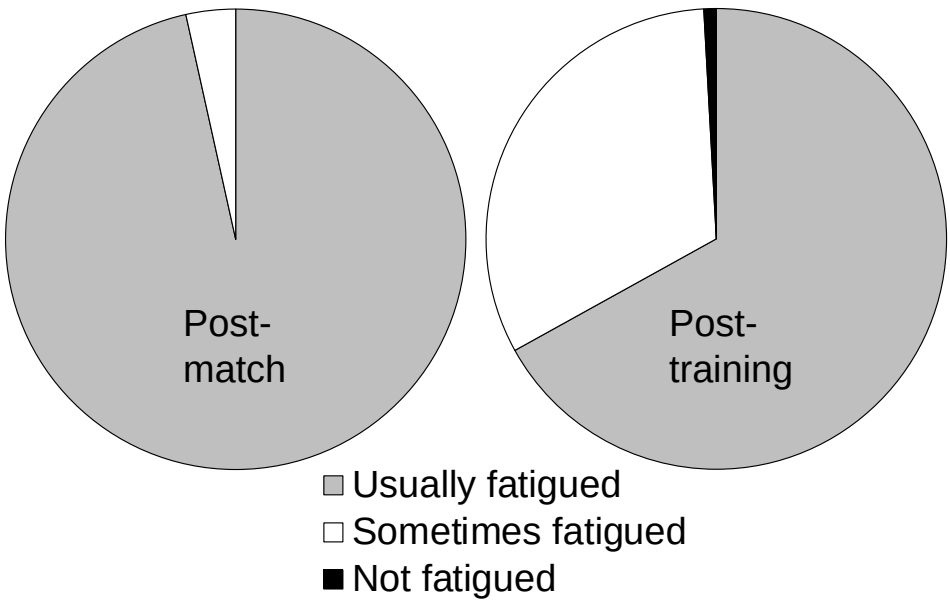

Figure 4.1. Respondent frequency (%) of perceptions of fatigue occurrence following match and training activity.

The perceived symptoms of fatigue by athletes and non-athletes (coaches and practitioners) are shown in Table 4.3. More than 10 % difference in the respondent frequency of perceived symptoms was shown between the two groups for six out of eleven symptoms post-training, and four out of eleven symptoms post-match. Mann-Whitney Tests revealed statistical trends ($0.05 < P < 0.10$) in these group differences for post-match physical weakness and reduced sleep time; and post-training pain, physical weakness, increased sleep quality. A significant ($Z = -2.038$, $P = 0.042$) difference was found for post-training task thinking time.

In terms of between-match recovery, 62 % of all respondents felt that recovery was 'frequently' or 'always' reached, with 12 % either 'rarely' or 'never' recovering. A Wilcoxon Signed Ranks Test showed that this profile was significantly different to between-training session recovery, where only 24 % felt that recovery was 'frequently' or 'always' reached, and an increase to 38 % either 'rarely' or 'never' recovering ($Z = -6.703$, $P < 0.0005$). Mann-Whitney Tests revealed no difference between perceptions of athletes and non-athletes across the between-match and between-training timeframes (Between-match $P = 0.132$, between-training $P = 0.757$).

Time of day appeared to influence perceived occurrence of fatigue and difficulty of recovery (Figure 4.2). Post-training fatigue was perceived by a similar number of respondents following morning, afternoon, and evening sessions. However, the challenge of recovery was markedly greater following evening training and matches. Post-match fatigue was perceived to be much greater than that following morning and afternoon fixtures. The main reasons supporting the challenge of recovery following evening matches was heavily in favour of the effects upon sleep (62 %). Following evening training sessions 72 % reported that challenged recovery was due to less recovery time before the next session, less potential for adequate sleep, and the added impact of the day's activities prior to evening training.

Table 4.2. Respondent frequency (%) of perceived reasoning for feeling fatigue following match and training activity across respondent groups; and mean ± SD days taken to fully recover from matches.

	Coach (n = 4)		Medical (n = 26)		Sp Sci / S&C (n = 7)		Athlete (n = 78)	
Main reasons for fatigue following activity	**Match**	**Training**	**Match**	**Training**	**Match**	**Training**	**Match**	**Training**
Damage to the body	75%	50%	73%	27%	86%	43%	87%	50%
Physical tiredness	100%	75%	100%	92%	100%	100%	100%	96%
Mental Tiredness	75%	25%	88%	65%	86%	57%	62%	54%
Perceived time to full recovery post-match (days)	3.0 ± 0.7		3.0 ± 0.5		2.6 ± 0.5		3.0 ± 0.9	

Table 4.3. Frequency (%) of respondents' perceptions of fatigue symptoms after matches and training. Figures in blue represent >10 % difference in group perception after matches, figures in red represent >10 % difference in group perception after training. Figures in *italics* represent a trend for (P < 0.10) difference in group perception of symptom. Figures in **bold** represent statistical significance (P < 0.05).

	After matches			After training		
Perceived fatigue symptoms	**Athlete**	**Practitioner / Coach**	**diff P value**	**Athlete**	**Practitioner / Coach**	**diff P value**
Muscle Soreness	94%	86%	.389	94%	86%	.191
Pain	76%	73%	.941	50%	35%	*.098*
Reduced Wellness	59%	73%	.175	50%	62%	.168
Physical Weakness	76%	59%	*.062*	56%	38%	*.083*
Reduced sleep quality	78%	86%	.173	17%	22%	.718
Increased sleep quality	9%	11%	.911	49%	30%	*.070*
Reduced sleep time	67%	81%	*.067*	13%	14%	.797
Increased sleep time	10%	16%	.572	41%	32%	.435
Increased thinking/task time	29%	35%	.680	32%	14%	**.042**
Increased irritability	59%	57%	.949	37%	38%	.913
Increased stress levels	55%	70%	.149	38%	27%	.269

Monitoring fatigue and recovery (n = 115)

The majority of respondents (91 %) reported the use of Global Positioning Systems (GPS) data, heart rate monitors (72 %), physical tests (77 %, and self-reporting scales (78 %). Only 8 and 10% had experienced saliva and blood sampling for monitoring recovery, respectively. As such, around 50 % weren't sure of their usefulness in monitoring fatigue and recovery status, whereas self-reporting scales (79 %), physical tests (86 %) and GPS (82 %) were collectively rated as either 'quite useful' or 'very useful'.

Cold therapies (n = 115)

The majority of respondents were familiar in experiencing or applying cold water immersion (CWI) and cold compression devices (CCD) for recovery (87 % and 84 %, respectively). Only 56 % reported that they had experienced or applied WBC as a recovery modality. A multi-answer question explored perceived benefits of all three cold therapies and is shown in Figure 4.3. Despite a plethora of benefits reported, 94 % and 97 % of respondents still displayed uncertainty around the benefits of WBC and CWI, respectively. Only 6 % of respondents expressed uncertainty in the effects of CCD.

Whole-body cryotherapy

58.3 % (n = 67) of respondents reported to have experienced or prescribed WBC; 65.7 % (n = 44) of these expressed its use after both match and training environments, 26.9 % (n = 18) reported post-match only, and 7.5% (n = 5), post-training only.

Table 4.4 displays the treatment parameters experienced or applied in practice. Following matches, most had used WBC within one hour of match completion (72.7 %, n = 48). Nearly half had used WBC the following day (47.7 %). A single exposure of WBC was the most common treatment dosage in a WBC session (53.8 %, n = 35), however, two (33.8 %, n = 22) and three (41.5 %, n = 27) repeated exposures in one treatment session were also reported.

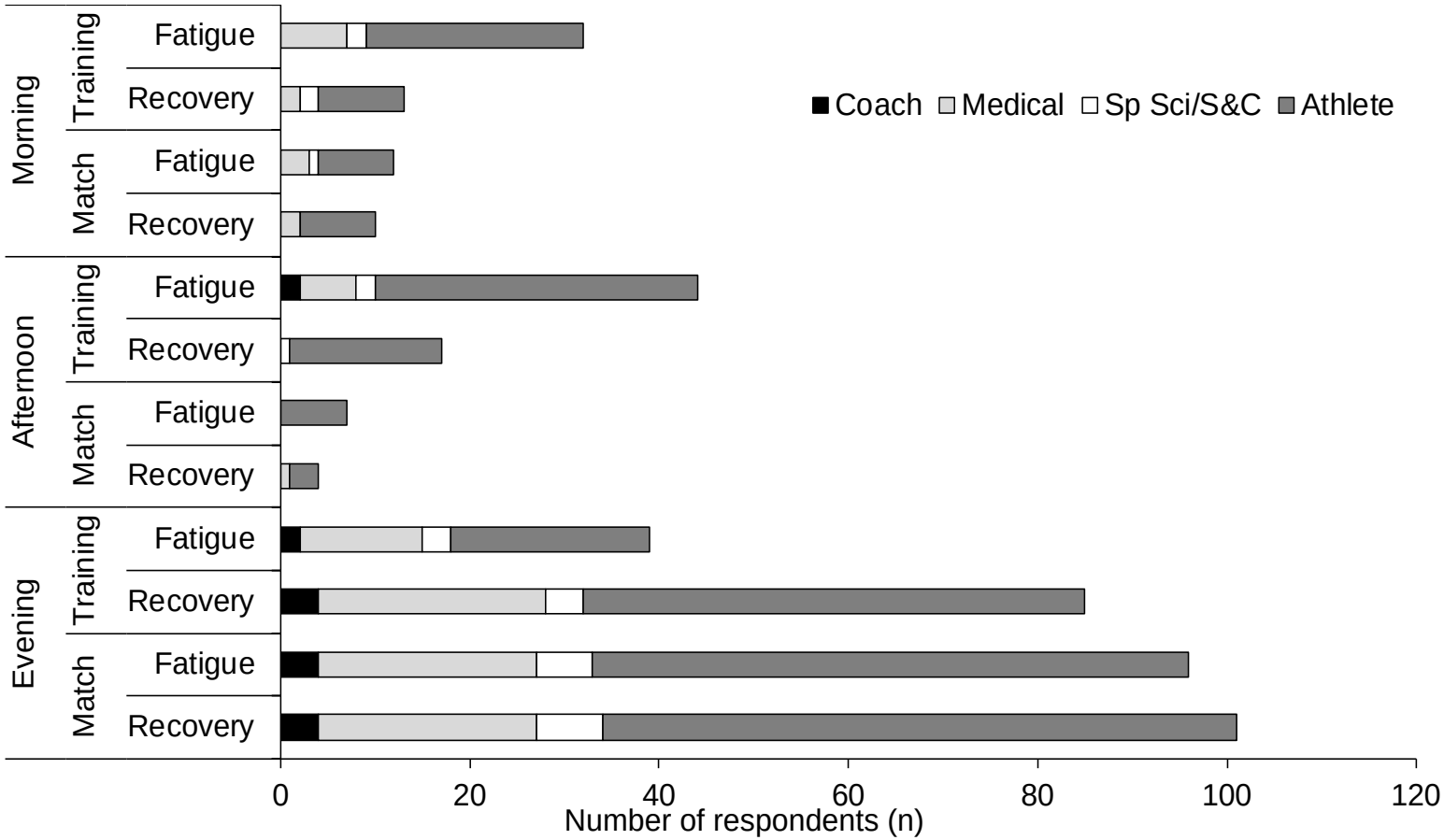

Figure 4.2. Respondent frequency (n) of the greatest perceived level of fatigue and difficulty of recovery subject to the time of day of match or training conditions.

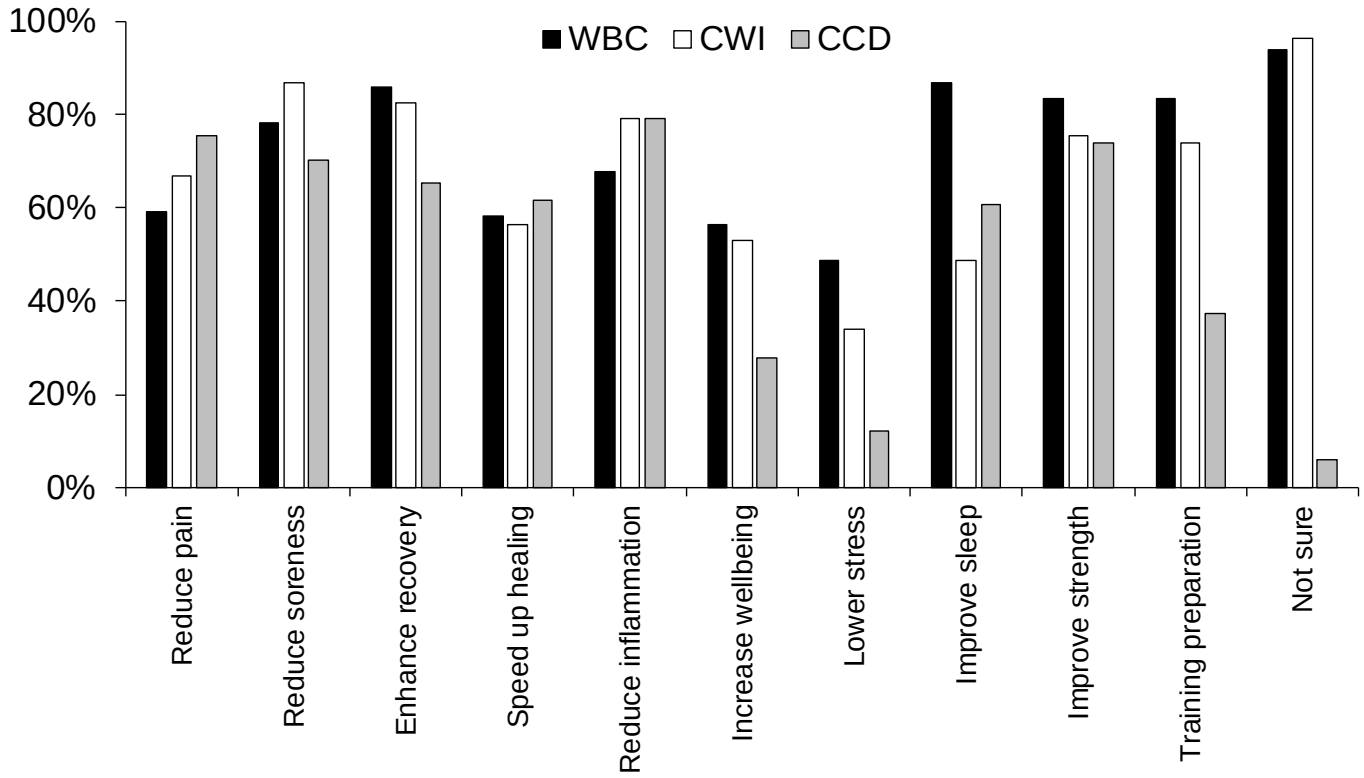

Figure 4.3. Respondent frequency (%) of perceived benefits of cold therapies for recovery.

Table 4.4. Summary of the timing, frequency and exposure dosage of WBC as experienced or applied by respondents.

Frequency		Timing		Exposure dosage	
Used after most matches	17.9%	< 1h	100.0%	Single	53.8%
Used after most training sessions	4.5%				
Used once or twice monthly	6.0%	1 - 12 h	38.8%	Twice	33.8%
Infrequent use	53.7%	>12 h	47.7%	More than twice	41.5%
One-off use	23.9%				

A Wilcoxon Signed Ranks Test revealed that perceived effectiveness of WBC was significantly greater after matches than after training (Z = -2.491, P = 0.013, see Figure 4.4). 76.5 % of respondents in this group felt that WBC made recovery faster. The remainder (23.5 %) felt that WBC made no difference to recovery time.

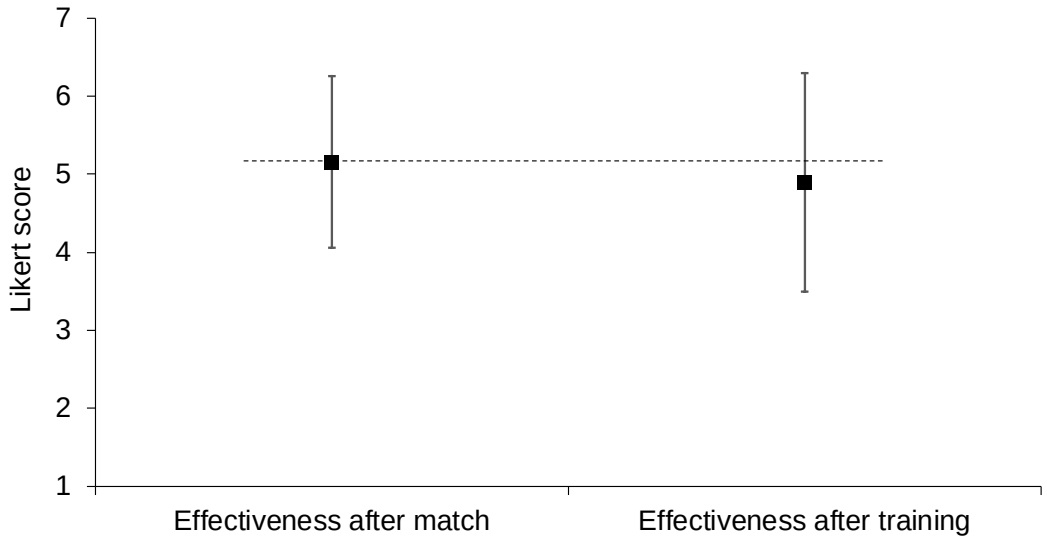

Figure 4.4. Mean ± SD of 7-pt Likert score for perceived effectiveness of WBC. Dotted line provides a means of visual comparison of average score (1 = not effective, 7 = highly effective).

The most common beliefs behind the use of exposure dosages (whether experienced or prescribed), are shown in Table 4.5.

Forty respondents answered a voluntary, open question allowing positive and / or negative experiences of WBC to be reported. From this group 77.5 % (n = 31) reported positive experiences where 32. 6% (n = 14) reported benefits to sleep, 27.9 % (n = 12) reported psychological benefits and 7 % reported reduced soreness (n = 3). Negative experiences (7.5%, n = 3) reflected discomfort with the extreme cold exposure, and other responses (15%, n = 6) were identified as barriers to use, for example logistical issues, having to wear protective equipment, and being unaware of the modality itself.

Eighty-six participants provided responses which pertained to their intended use of WBC given the opportunity. This group of responses was drawn from those who had not experienced WBC, and also those who had either used WBC infrequently or on a one-off occasion. A Wilcoxon Signed Rank Test revealed that respondents would be significantly more likely to adopt WBC post-match than post-training ($z = -4.382$, $P < 0.0005$, see Figure 4.5). The most common implementation methods of the group who would use WBC are presented in Table 4.6. A repeated exposure dosage applied within 1 h of activity, two – five times per-week was the most common combined choices of implementation of WBC. A total of 15 % (n = 13) of respondents (1 coach, 1 medical, 1 Sp Sci / S&C and 10 athletes) reported that they would not use WBC given the choice.

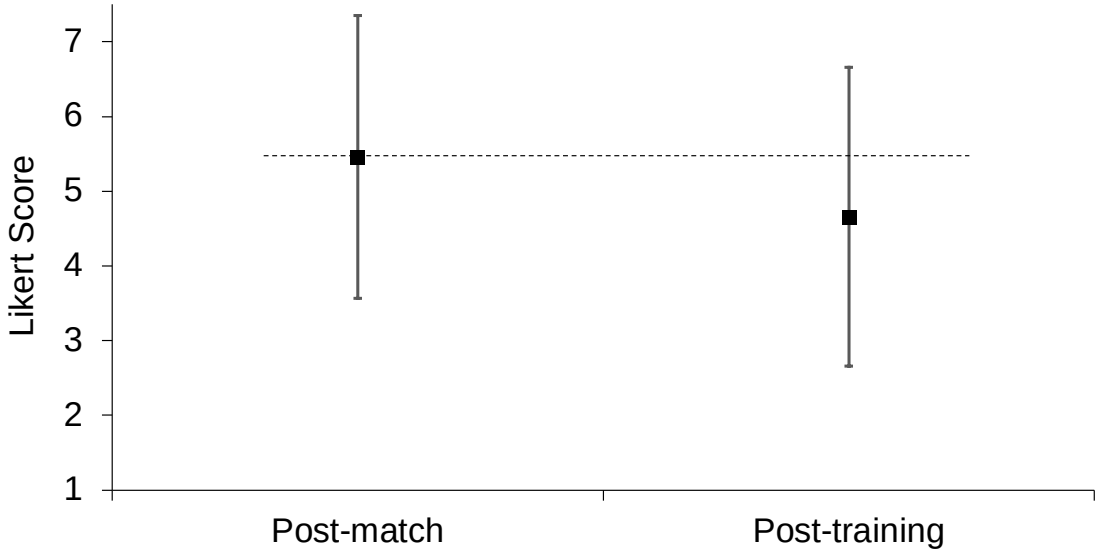

Figure 4.5. Mean ± SD of 7-pt Likert score for likelihood of adoption of WBC. Dotted line provides a means of visual comparison of average score (1 = not at all likely, 7 = highly likely).

Table 4.5. Common reasons for the frequency and exposure dosage of WBC experienced or applied in practice

Parameter		Reason	Number of respondents	
Exposure dosage		Research informed at the time of use	Once = 5 Twice = 1	> Twice = 6
		Told it was best way / most adequate dose	Once = 5 Twice = 3	> Twice = 1
		Don’t know	Once = 7 Twice = 5	> Twice = 4
		Due to its perceived effects / benefits	Once = 6 Twice = 5	> Twice = 12
		Budgets/cost	Once = 2 Twice = 0	> Twice = 0
Frequency	Less frequent	Cost / logistics / access	24	
		Unsure of / lack of perceived benefits	4	
	More frequent	Beneficial in congested fixtures	10	
		Beneficial for enhanced recovery	12	

Table 4.6. Respondents' (n = 86) ideal parameters of WBC implementation.

Frequency		Timing		Exposure dosage	
Once daily	19.5%	Within 1 hour	53.5%	Once	32.5%
2-5 times per week	41.5%	Between 1 and 12 hours	29.1%	Twice	41.3%
Once per week	15.9%	After 12 hours	3.5%	More than twice	10.0%
1 - 3 per month	8.5%				

Discussion

This is the first study to investigate the beliefs, perceptions and practice of WBC in an elite contact sport context. This investigation has identified a number of important considerations in relation to the context of this thesis, in terms of perceptions of fatigue status and the use of WBC for recovery.

Use, perception and beliefs of WBC when used as a recovery modality

Only 58.3 % of the respondent group had experienced or used WBC for recovery purposes, and only 22.4 % adopted the use of WBC on a regular basis. Given that recent publications report an increase in its popularity amongst elite athletes (Lombardi et al., 2017; Patel et al., 2019), a clear discrepancy in the low use of WBC has been evidenced in this group of respondents across rugby league, rugby union and football teams. Its apparent 'popularity' is perhaps expressed by the opinion that a high (85%) proportion of respondents would adopt WBC in practice given the opportunity, furthered by a strong belief in supporting its potential benefits in enhancing recovery. These appear to be heavily surrounding sleep benefits, especially in comparison to CWI and CCDs (Figure 4.3), however, a high level of

uncertainly as to its overall benefits and mechanisms is also evident. Despite this, those who had experienced WBC, frequently reported a strong influence upon sleep particularly after evening matches, where it appeared to provide the greatest perceived benefits. This was a significant finding in this study when post-training and post-match use was compared ($P = 0.013$). As a result, respondents were significantly more likely to adopt WBC practices post-match than post-training ($P < 0.0005$).

In regard to the practice of WBC, respondents were most likely to use WBC within the short term (<1 h) post activity, and regularly (2 – 5 days per-week). However, many respondents were unaware of the reasoning for choosing or experiencing WBC prescription parameters (exposure dosage, frequency). In this regard, only 12 respondents felt that they had followed an evidence-based approach in their application of WBC. As such, it is necessary that future research addresses more practice-based questions relating to the parameters of application so that practice can be suitably informed.

Given the lack of directly related data in this field, it is difficult to make comparative assumptions about the practices of WBC. However, the behavioural choices and reasons underpinning the use (or lack of use) of recovery strategies does show similar patterns to previous studies. Athletes still rely on information and guidance from coaches and practitioners to inform the use of modalities, since some athletes reported being 'told to use WBC' as a reason for use and/or the parameters of use. In the case of this study, some practitioners felt guided by the WBC providers' information, as well as recommendations from other practitioners and the research available. What remains common with other studies exploring recovery modalities, education of athletes, coaches, and in the case of WBC, medical and sport science practitioners is required to establish a more reasoned approach to the use of WBC (Murray et al., 2016; Crowther et al., 2017; Murray et al., 2018). Van Wyk & Lambert (2009) express the importance of fully informing players of recovery strategy protocols, processes, and scientific basis so that they can assume some personal responsibility in more accurate choices for adopting a recovery method within a club routine.

Perception of fatigue occurrence, symptoms, monitoring, and recovery

Subjective measures and perceptions were strongly linked with an athlete's status of fatigue, and can therefore be used with confidence (Saw et al., 2015). As such the responses of athletes in this study should provide insight to how the perception of fatigue from a non-athlete perspective (i.e., the coach or practitioner) requires attention. This study indicated that non-athletes showed the potential to underplay the level of fatigue experienced following training sessions. In some ratings of post-activity fatigue symptoms, there was >10 % discrepancy between athletes and non-athletes in the beliefs of what symptoms were occurring (for example, pain, weakness, stress levels, cognitive function), particularly after training sessions. Athlete responses indicate that less muscle damage and mental fatigue is reported after training, however, non-athletes should be aware of the cumulative nature of fatigue when regular training is part of the athlete's schedule, despite agreeing a similar time frame of recovery from matches of approximately three days before quality high intensity work is feasible.

This is particularly important when time of day of training is considered. The belief that recovery is challenged more from evening activity due to negative effects upon sleep is evidenced here. Poor sleep quality and lowered time to recover before the next session is a view corroborated by previous studies and has been clearly highlighted as a fatigue risk factor (Gupta et al., 2017). Time of day of training or competition is therefore an important consideration when accounting for the perceived benefits of WBC as highlighted in the data above, and the optimisation of its prescription.

In terms of monitoring fatigue, biochemical and salivary analyses were not frequently used or experienced across the respondents, and their usefulness was largely unknown. In the live sport environment, this appears to be a consistent theme with previous research, mainly due to factors affecting accessibility, cost and analysis time (Taylor et al., 2012). Similar to previous data, the respondents in this study expressed that fatigue and recovery were more likely to be monitored through GPS, perceived ratings and physical tests. Whilst this remains useful, researchers should continue to broaden the understanding in responses of biological markers to team sport activity, in order to understand the mechanisms underpinning an overall athlete perception (Slimani et al., 2018).

Limitations

In order to gain a clearer view across contact sports and practitioners, greater participant numbers are still needed. There was large disparity in groups sizes, therefore non-athlete groups had to be combined for most comparative analyses. Therefore, it remains unknown as to whether perceptions differ between team staff roles such as coaches *vs* medical practitioners. Given that participants from individual teams did complete the questionnaire, the adoption of practice and implementation of WBC data in this study may be skewed towards how a particular team operates its recovery practice and may not be representative of other teams competing in the same sport. As such, the highlighted issue of cost and accessibility is likely to influence the priority choices for recovery interventions of teams. This has been reported elsewhere in regard to the use of specific recovery modalities such as cold or water-based therapies (Crowther et al., 2017).

The data collected mostly represented a rugby league population in terms of athletes (85 %), and from a staff perspective (49 %). This is likely to cause bias within the data in regard to perceptions of fatigue and recovery in light of the varying sport demands across rugby league, union, and football.

When considering questionnaire completion rate, some non-mandatory, open questions exposed greater chance of some fields not being answered; this was unavoidable in this instance due to post-question instructions and question navigation in the final section addressing WBC. However, this only occurred (< 10 % of eligible question respondents) in questions relating to beliefs on WBC exposure dosage and timing of WBC application where open text was required.

Finally, from a perspective of questionnaire design, a formal Delphi scoring system and Cronbach's alpha were not used and so this may be viewed as a limitation of consistency of question responses. Two pilot rounds of the survey were provided, however only qualitative feedback regarding question design and clarity was collected.

Conclusion

This study aimed to investigate the beliefs, perceptions of athletes and practitioners in the practice of WBC in elite contact sports. An online survey gathered information from rugby league, union, and football populations; the findings of which may help inform the understanding of athletes' fatigue states and recovery following training and matches. Firstly, practitioners may not accurately perceive the extent and nature of the fatigued state of athletes. Therefore, accurate and agreeable methods of monitoring should look to draw viewpoints together using subjective and objective means. Recovery is challenged by evening activity more so than that in the morning or afternoon, particularly from matches, which appears to be driven by poorer sleep quality and less time to recover. With regards to extreme cold therapy, the adoption of WBC practice is low in this particular group, and implementation methods are variable and generally uniformed. However, the effects of WBC are perceived to be very positive, and the potential adoption for WBC is high, particularly if cost and accessibility barriers to WBC were reduced. This study identified a strong belief that WBC can benefit recovery, mainly by a perceived positive influence on sleep. However, this may only be based upon perceptions from infrequent experiences of WBC, or anecdotal information passed between athletes and/or practitioners.

Chapter 5

The post-match and post-training response of endocrine markers to whole-body cryotherapy during a typical between-match recovery period in an elite rugby league team.

Introduction

Whole-body cryotherapy (WBC) involves short exposures of up to three minutes in a cooled air chamber, in temperatures between between -110 to -135 °C. It is used to enhance athlete recovery following training and competition, in order to optimise the internal environment for subsequent maximal physical performance.

Short-term benefits of WBC such as improved sleep quality, perceived recovery and time-trial performances have been reported; when compared to regular recovery procedures in synchronised swimmers across a 14-day intensified training period (Schaal et al., 2015). The alteration in endocrine profile by daily WBC application during training and competition is sparse. One such study by Grasso et al. (2014) investigated National-level rugby union players across a two-week training period and observed an increase in salivary testosterone (T) and reduction in salivary cortisol (C) at the end of the two-week period, after players experienced morning and afternoon sessions of WBC. However, a control group was not employed, and samples were only obtained at the extremes of the training period.

The systematic review of this thesis highlighted the lack of research in regard to the endocrinological responses to WBC when applied to periods of live, competitive high intensity collision sport. Hormonal homeostasis, particularly in context of an individual's anabolic and catabolic status, is an important component of recovery monitoring. Being able to indirectly observe the nature of the post-match stress response via C concentrations, and signalling for tissue adaptations via T concentrations during the recovery period allows practitioners scope for improved recovery and training strategies. Only McLellan et al. (2010) have published data observing the endocrine response following a rugby league match and indicated that at a 48-h period is required for hormonal normalisation. However, the effects of WBC have not yet been studied during a complete between-match period to analyse any impact on hormonal values during a post-match recovery process. Amongst the aforementioned WBC studies, no intermediary observations have been recorded and so the data reflects a start and end snapshot of the measurement period. As such, additional measures would be useful to express any pattern of change in hormonal response.

The aim of this study was to observe the post-match and post-training endocrinological response to WBC sessions used across a seven-day turnaround competitive period. In light of the work by Grasso et al. (2014) and Schaal et al. (2015), and the systematic review of this thesis, the working hypotheses were as follows: (i) the post-match hormone response would be influenced by frequent use of WBC during the recovery period; (ii) the use of WBC would produce a different response when used post-match or post-training due to the difference in the overall fatigue stimulus.

Method

Participants

Eighteen elite RL players, of mean ± SD age 27.6 ± 4.7 years; height 1.83 ± 0.10 m and mass 97.0 ± 9.9 kg volunteered to participate in the study. Informed consent was obtained from all participants, and, prior to the study, all participants were briefed on the purpose, procedures, and benefits of the study. The study was approved by the University of Bolton Research Ethics Committee. Data from twelve players were observed across all time points and therefore were included in the analysis. Player samples were excluded if they reported an injury following the initial match during the sample period or were not selected for the subsequent match.

Experimental Approach

Elite RL players from a European Super League team were monitored *via* saliva sampling over a nine-day period within the final 6 weeks of a competitive season. The sample period (Figure 5.1) involved two matches, separated by seven days (M1+0 and M2+0), WBC treatment exposures on days M1+1, M1+3, M1+5, and M2+1, and saliva sample collections before and after WBC sessions on M1+1, M1+3, and M2+1.

WBC Procedures

A mobile trailer-mounted liquid nitrogen cryochamber (Sappari, UK), owned and operated by British Oxygen Company Linde (BOC) was used for all WBC treatments.

All participants consented to the use of the cryochamber according to the guidelines set by BOC and were cleared of any contraindications according to the BOC mandate by the club doctor. Participants donned the appropriate safety attire consisting of shorts, thick socks, sandals, hat, gloves, elbow and knee joint elasticated bandage coverings, and face mask covering the nose and mouth. Participants entered the cryochamber *via* an initial acclimatisation chamber cooled to -60 °C for 30 s. They then entered the main chamber cooled to between -120 and -135 °C for three minutes. These application parameters are recommended in order to create sufficient thermal gradients following removal of the WBC stimulus (Westerlund et al., 2003). Once complete, the participants stood in external environmental conditions (recorded range 14 to 18 °C) for five minutes before re-entering the WBC chamber for a second treatment exposure. The second dose was provided given the understanding that poor thermal conductivity of air to skin, particularly in lean, athletic males may limit the effects of WBC in this population group (Hammond et al., 2014).

Saliva sampling and analysis procedures

Saliva was chosen as the medium for obtaining C and T hormone concentrations due to the non-invasive nature and relative ease of sample collection compared to blood sampling. Saliva samples were collected as per the schematic in Figure 5.1. It was required that the participants refrained from eating or drinking in the hour prior to sample collection in order to ensure regular salivary content and quality.

For all samples taken, water was provided five to ten minutes prior to sample collection to rinse the mouth. It was only upon rigorous adherence to these steps that a sample was considered suitable, and reliable, for the purpose of analysis. Salivette oral swabs (Salimetrics LLC, State College, PA, USA) were used to collect unstimulated saliva, placed beneath the tongue for three to four minutes. The salivettes were then placed in individual storage tubes (Salimetrics LLC), immediately placed on ice and frozen within one hour at <-20 °C until required for analysis. Samples were labelled with individual coding so that participant data were pseudonymised and allowed for a single-blind sample analysis process.

For analysis preparation, samples were defrosted and centrifuged at 5600 rpm for five minutes. All saliva samples deemed reliable upon collection, and also deemed usable following sample analysis, were included in the statistical data presented. C (µg/dl) and T (pg/ml) were analysed in duplicate using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics LLC). All samples were assayed and analysed by the study author. Microplate analyses were carried out using a microplate reader with Gen-5 software (ELx-800, Biotek Instruments Inc., Winooski, VT, USA). Standard curves were constructed as per the manufacturer's instructions, standards and sample controls (Salimetrics, LLC).

T/C ratios were determined by dividing the concentration of T by the concentration of C. The manufacturer-determined minimum detection limit for T was 1.0 pg/ml (relative 0.0001 µg/dl), with an average intra- and inter-assay coefficient of variance (CV) of 4.1 ± 4.7 % and <12 % respectively as calculated by the study author. The minimum detection limit for C was 0.007 µg/dl (relative 70 pg/ml), with average intra- and inter-assay CV of 4.3 ± 4.0 % and <12 % respectively. CV was calculated per duplicate sample during microplate analysis and provided by the Gen-5 data analysis output.

Statistical Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL), IBM version 26. The normality of distribution was checked using the Shapiro-Wilk test. Differences between the short-term effects of WBC when used post-match and post-training during the sample period were evaluated using a general linear model (GLM) with repeated measures, within subject factor 'condition' (three levels: 1 = post-match without previous WBC influence, M1+1; 2 = post-training with previous WBC influence, M1+3; and, 3 = post-match with previous WBC influence, M2+1) and within subject factor 'time' (two levels: pre- and post-WBC); and interaction. To correct violations of sphericity, the degrees of freedom were corrected using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate. Effect sizes (ES) were calculated from the ratio of the mean difference to the pooled standard deviation. The magnitude of ES was classified as trivial (≤0.20), small (>0.20-0.60), moderate (>0.60-1.20), large (>1.20-2.00), and very

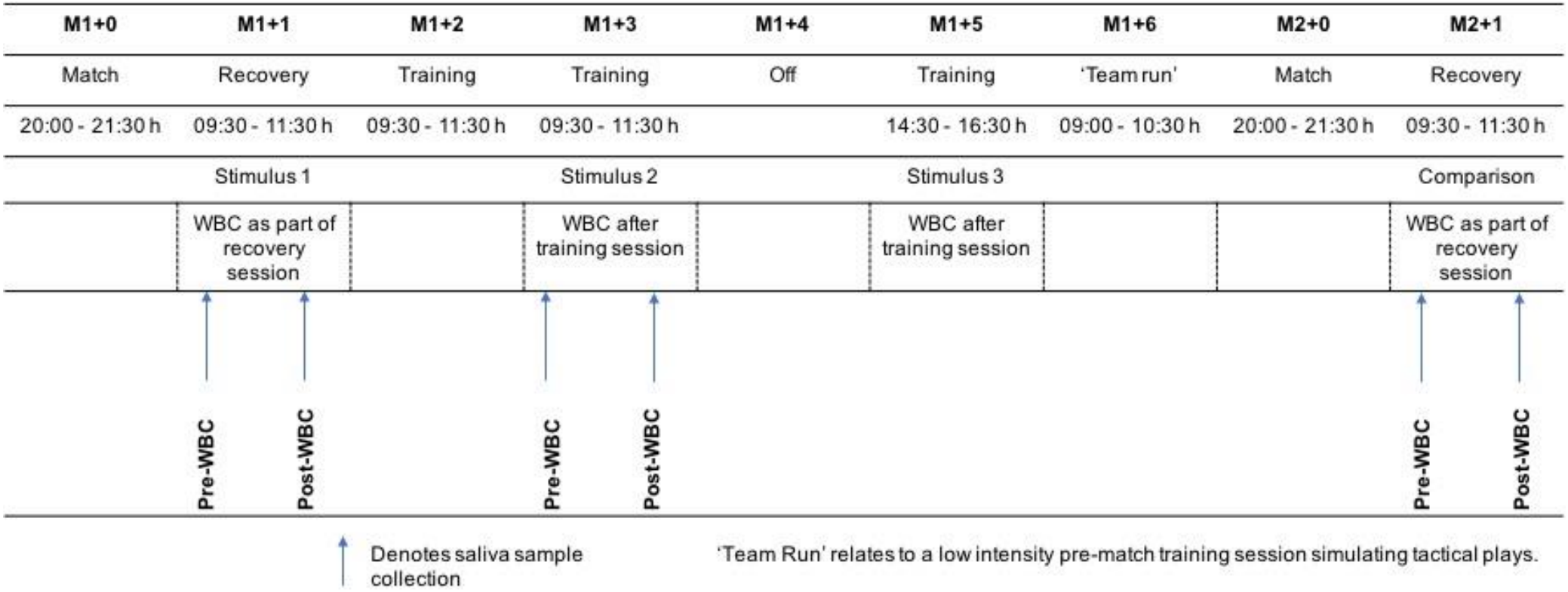

Figure 5.1. Schematic for WBC and sampling over the nine-day period.

large (>2.00) based on the guidelines from Batterham and Hopkins (2006). All data remained in original scale measures for statistical analyses and presented as mean ± SD unless otherwise stated. Statistical significance was set at an alpha level of 5% ($P < 0.05$). When the SPSS output demonstrated significance levels of $P = 0.000$, these were corrected to $P < 0.0005$ (Kinnear & Gray, 2006). A statistical trend was identified with $0.1 > P > 0.05$.

Results

Data for twelve players were included in the analysis and is shown in Table 1. Data from six players were excluded due to inconsistent team selection in each match and therefore salivary samples were not available.

Table 5.1. Mean ± SD values for hormonal markers at all measured time points and conditions.

	Pre-WBC	Post-WBC
T (pg/ml) (n=12)		
M1+1	39.3 ± 17.0	46.6 ± 17.9
M1+3	53.2 ± 7.4	45.4 ± 7.8
M2+1	24.2 ± 11.8	31.8 ± 14.6
C (µg/dl) (n=12)		
M1+1	0.61 ± 0.33	0.48 ± 0.27
M1+3	0.27 ± 0.10	0.25 ± 0.10
M2+1	0.33 ± 0.12	0.31 ± 0.16
T/C ratio (n=12)		
M1+1	77.5 ± 37.7	123.3 ± 61.3
M1+3	228.2 ± 91.8	203.4 ± 71.8
M2+1	74.1 ± 19.2	109.4 ± 40.0

Cortisol response

For the analysis of the acute C responses the GLM revealed a statistically significant effect for condition ($F_{1.9, 20.4} = 8.7$, $P = 0.002$). Pairwise comparisons showed that the M1+1 condition C values were significantly higher than at M2+1 (mean difference =

0.22 µg/dl, 95% CI = 0.00 - 0.44, P = 0.046; ES = 0.86) and at M1+3 (mean difference = 0.29 µg/dl, 95% CI = 0.05 – 0.52, P = 0.016; ES = 1.08), respectively. There was no statistical difference between M2+1 and M1+3 (mean difference = 0.07 µg/dl, P = 0.710). Whilst there was no significant effect for time between pre (0.40 ± 0.26 µg/dl) and post (0.35 ± 0.21 µg/dl) values, a statistical trend was evident ($F_{1, 11} = 3.3$, P = 0.096). No interaction effect was established, such that the profiles for the three conditions declined in a similar pattern ($F_{1.2, 13.1} = 0.99$, P = 0.353; see Figure 5.2).

Testosterone response

For the analysis of the acute T responses the GLM revealed a significant effect for condition ($F_{2, 22} = 21.0$, P < 0.0005). Pairwise comparisons showed that the overall T concentrations at M2+1 were significantly lower (mean difference = 14.9 pg/ml, 95% CI = 6.3 - 23.5, P = 0.001: ES = 0.87) values than at M1+1. The values at M3+1 was significantly higher than those at M2+1 (Mean difference = 21.5 pg/ml, 95% CI = 10.5 – 32.4, P = 0.001: ES = 1.38). There was no statistical difference between M1+1 and M1+3 (P = 0.198). There was no significant effect for time where pre-post values were similar ($F_{1, 11} = 0.7$, P = 0.416). There was a trend shown in the interaction effect ($F_{1.4, 15.7} = 3.4$, P = 0.074; see Figure 5.3). This is shown by the decreasing T concentrations pre- to post-WBC during M1+3, whereas the T values increased pre- to -post-WBC during both post-match conditions.

Testosterone/Cortisol relationship response

For the analysis of the acute [sTesto/C ratio responses the GLM revealed a significant effect for condition ($F_{1.6, 17.5} = 27.5$, P < 0.0005). Pairwise comparisons showed that the ratio at M1+3 was significantly higher than that observed at M1+1 (mean difference = 115.4, 95% CI = 58.8 – 172.0, P < 0.0005; ES = 1.28) and M2+1 (mean difference = 124.0, 95% CI = 60.5 – 187.5, P = 0.001; ES = 1.40). There was no statistical difference between M1+1 and M2+1 (P = 1.000). There was a significant effect for time ($F_{1, 11} = 10.3$, P = 0.008), where pairwise comparison showed the overall pre-WBC value was lower than the overall post-WBC ratio (mean difference = 18.8, 95% CI = 5.9 – 31.6, P = 0.008: ES = 0.23). There was a significant interaction effect ($F_{2, 22} = 11.7$, P < 0.0005). Firstly, the WBC treatment appears to impact post-match and post-training situations inversely when compared to their respective pre-WBC values.

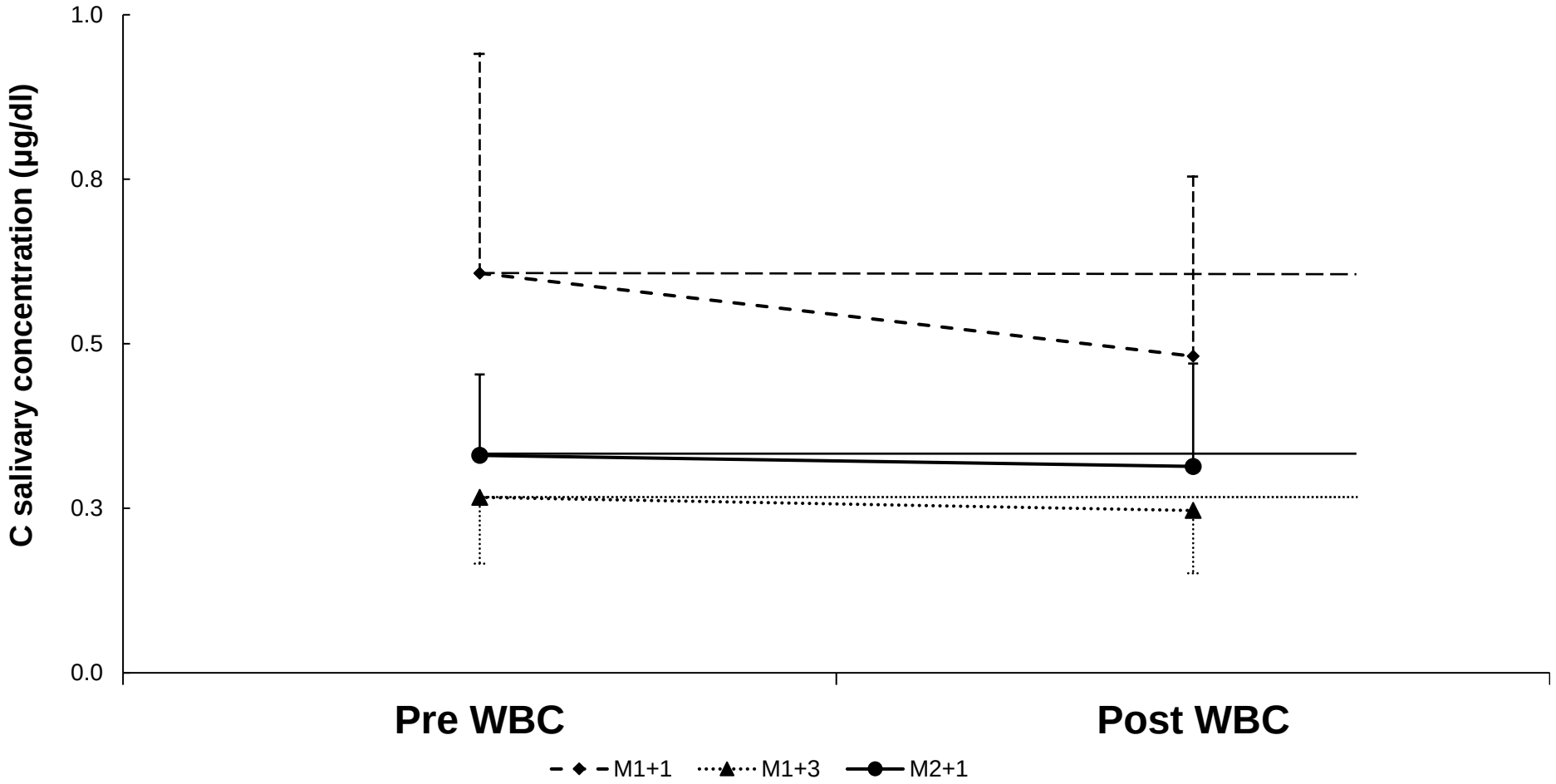

Figure 5.2: Mean ± SD C values for three different conditions of different exposures to WBC). Horizontal lines denote pre-WBC baseline values for comparison.

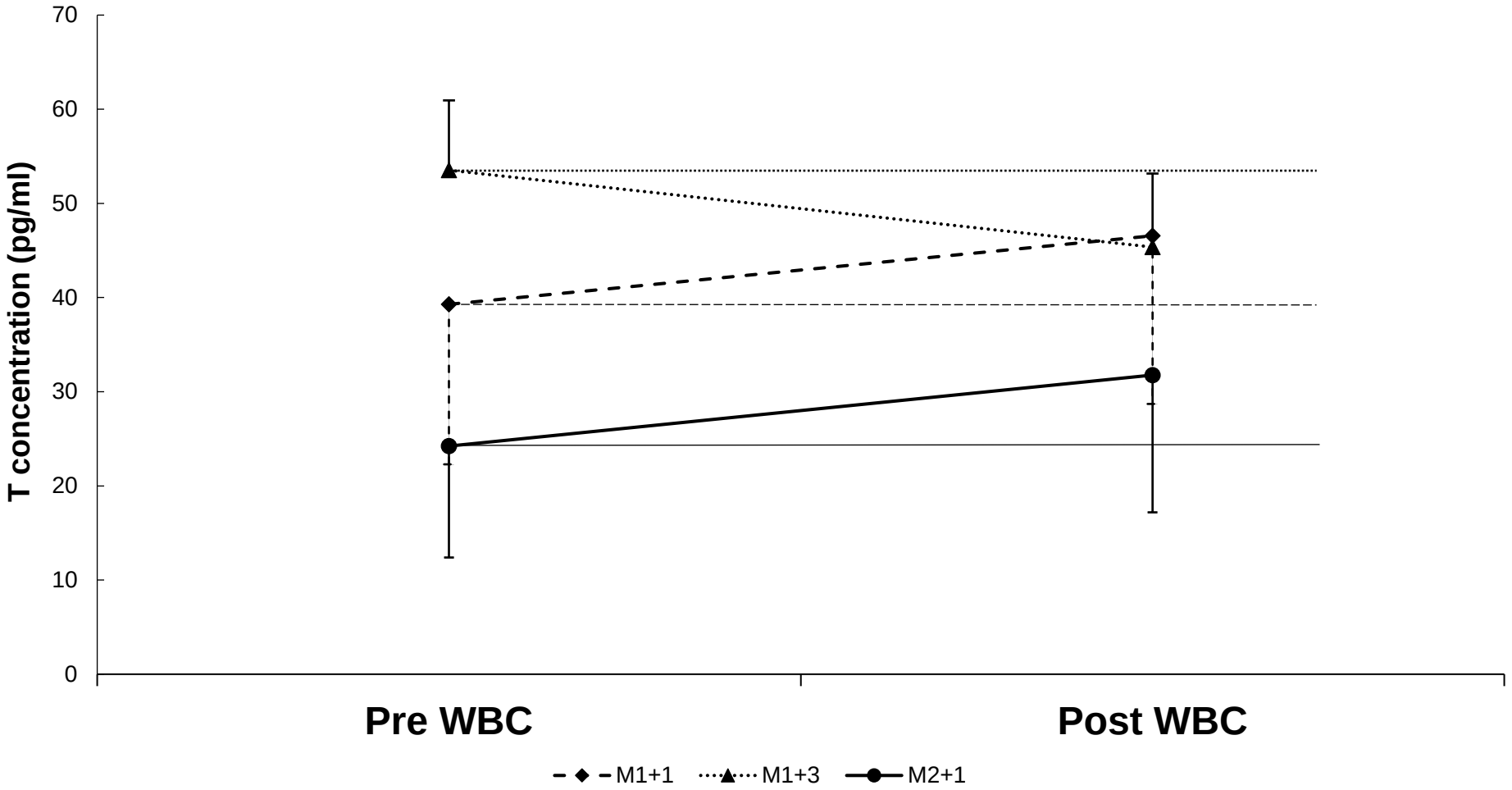

Figure 5.3: Mean ± SD T values for three different conditions of different exposures to WBC. Horizontal lines denote pre-WBC baseline value for comparison

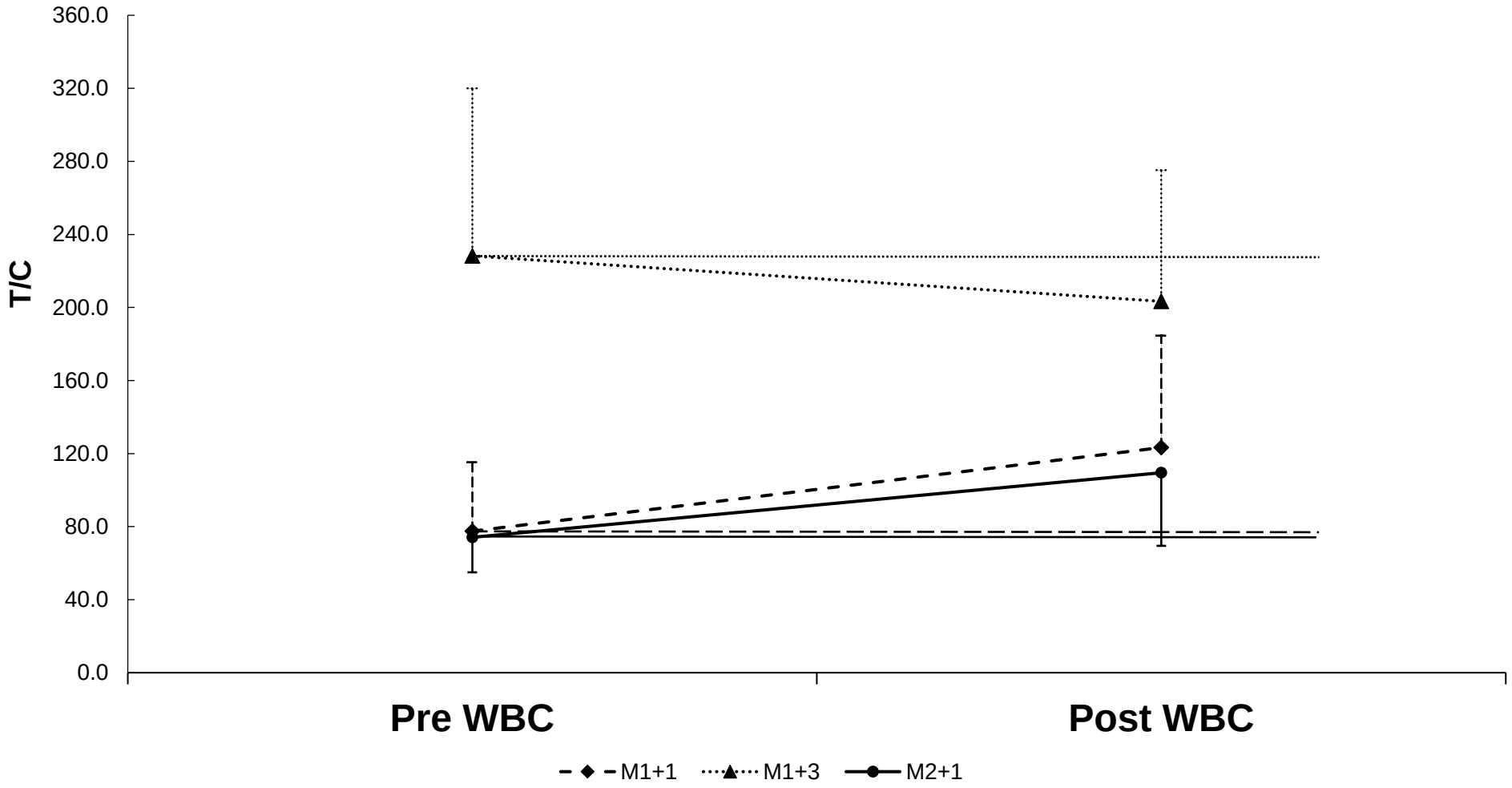

Figure 5.4: Mean ± SD T/C ratio values for three different conditions of different exposures to WBC. Horizontal lines denote pre-WBC baseline value for comparison.

Secondly, the ratio values observed during the training condition were notably elevated compared to that observed post-match, see Figure 5.4.

Discussion

This is the first study to observe the acute hormonal responses to the planned use of WBC in the context of an in-season, between-match recovery period in elite rugby league. Time points M1+1 and M2+1 were compared in order to address the first hypothesis i.e., to determine whether frequent use of WBC created alterations in hormonal profiling during a competitive period. When analysing the acute effect of WBC upon the post-match anabolic/catabolic profile, no difference in T/C ratio between first match (M1+1) and second match responses (M2+1) were shown (Figure 5.4). However, the significantly lower C (Figure 5.2) and T (Figure 5.3) concentrations observed post-match at M2+1, has two main explanations. Firstly, there may be the potential to support a short-term protective effect of the HPA axis which helps to mitigate the stress response during the first 12 h post-match i.e., at the point when the sample collection in this study occurred. This may be indicative of a dampening effect of the stress response, and beneficial to counter accumulated fatigue from match and training activity in a short time period. An acutely adaptive HPA, may, however, downregulate the resultant HPG response – which would effectively produce a reduced drive for post-match anabolic adaptations. A longer duration of sampling may be required to view the lasting effects of this block-use of WBC in the seven-day turnaround between two matches. The protective effect has been suggested by Grasso et al. (2014) who noted reduced C concentrations after a seven-day training period, with WBC administered twice per day. The authors suggested a potential short-term adaptive response within the HPA axis to the frequent use of WBC. Schaal et al. (2015) have also suggested that frequent use of WBC during a given training period may assist in the avoidance of fatigue accumulation and overreaching. Especially given the time in the rugby league season when the present data were collected (September is the final complete month of competitive activity), there may well be a short-term benefit to the repeated use of WBC at stages where increased fatigue can be identified during a rugby season (Alaphilippe et al., 2012). The two studies compared above applied WBC on a daily basis, whereas only three exposures to WBC were administered across the seven-day period in the present study. As such it may

be the case that multiple or compounding exposures may scale up the effects of the WBC stimulus when the stress response is warranted. The question of individual responses to consistent exposures also remains unanswered. In this regard, consideration should be given to body stature (Hammond et al., 2014) and fatigue status (Thorpe et al., 2017) in order to account for the need for therapeutic heat transfer and its effectiveness in a given situation.

The second explanation for the similar T/C ratio but lowered individual C and T concentrations could be related to the overall level of fatigue created during the match. In this instance, M1 could have been played at a greater intensity and overall, more demanding than M2. Measures of match intensity such as the number of high-speed collisions per player, or total distance travelled were not collected. Without such comparison between matches, it is difficult to ascertain the level of stress response generated by each match, and, as such, an 'easier' or less intense match may illicit a reduced stress response (Caetano Junior et al., 2017).

The second hypothesis of this study stated that WBC would produce a different hormonal response when used post-match or post-training. The acute responses of the T/C ratio to WBC in this study provide support for this statement in light of the significant interaction effect shown in Figure 5.4. In a post-match situation (at 12-h post-match, M1+1 and M2+1) the acute response to WBC increased the T/C ratio seemingly driven by increases in T concentrations (Figure 5.3). The opposite appears true when WBC is used post-training, at 60 h (M1+3) into the recovery period where the T/C ratio reduced following WBC application, driven by a downturn in T concentration. This suggests that timing of WBC is important when used during recovery periods. Whilst there appears to be a trend in lowering acute C concentrations, which, in isolation, may be beneficial, the impact upon T appears more variable. Hence, the driver of changes in the T/C ratio and relative anabolic or catabolic state looks to be largely determined by T concentrations, and as shown in this study, may be positively or negatively influenced by WBC. Naturally, given that sampling was restricted to 'spot' time points due to the nature of collecting data in a live competitive environment, any enhanced opportunity to perform broader time-course sampling would enable a clearer analysis of the observed changes.

A key issue faced in this study was the lack of a control trial or baseline measure. A catabolic relative state (to baseline measures) is expected post-match, which normalises through the post-match recovery period (Cunniffe et al., 2010; McLellan et al., 2010). What this study is unable to show is whether the use of WBC altered the rate in which the post-match increase in T and the T/C ratio occurred.

The T concentration after WBC at M1+3 showed an immediate significant decline, which questions whether WBC has any benefit upon anabolic hormonal balance when used in the midst of a recovery period. Two main reasons exist for these observations. The first could be that WBC is having no effect at all and that the changes observed would occur regardless. This is difficult to confirm given that there is no comparative period or initial 'rested' baseline, although data from McLellan et al. (2010) provides a means of comparison for recovery responses of the T/C ratio following a rugby league match albeit without any WBC influence. Their data shows that T/C ratio normalised and was at its most anabolic state at day three post-match, and then declined through day four and five. Despite a similar trend, the response shown in the present study could reflect the second reason for potential cause of change, i.e., WBC exposure during a training period downregulates the HPG axis depending on the preceding stimulus.

In either of the reasons given above, the evidence presented here suggests a counter-productive effect in the use of WBC post-training. Other studies also show either no, or harmful effects of WBC in some situations, typically after simulated laboratory experiments (Wilson et al., 2018; Kruger et al., 2019). Furthermore, this observation corroborates evidence of attenuated adaptive responses involving cold water immersion treatments used post-strength training for single session (Fuchs et al., 2020), and regular training exercise studies (Roberts et al., 2014).

In the context of post-rugby match recovery (McLellan et al., 2010), T responses have been shown to return to baseline pre-match levels within 24 h post-match, but then decline again from 96 h onwards following typical rugby-based and strength training sessions during this recovery period. This further supports the notion of potential interruption of the T response when WBC is used within a training period rather than post-match. Further, the observations recorded by McLellan et al. were taken in the

afternoon (15:30 – 16:30 h) and prior to evening training sessions whereas the data from the present study were mainly observed in the morning (08:30 – 09:30 h) before training sessions. Since T demonstrates a diurnal variation i.e., highest concentrations in the morning, and lowest in the evening, any comparisons made are subject to time of day interpretation. As such the 12 h post-match measure in this study may actually be lower still if measured at 24 h post-match.

In support of the anabolic upturn and increases in T in hormonal profile following WBC 12 h post-match, Russell et al. (2017) does support a short-term increase in T following WBC application. This was in academy footballers following maximal, repeated sprint activity, with WBC applied immediately after cessation of the repeated sprints. Within 2 h of WBC application T was elevated by 21 %, and by 24 h later was still elevated by 28 % over a baseline. The present study observed a 7.3 and 7.6 pg/ml increase in T across M1+1 and M2+1, respectively, a relative percentage increase of 16-24 % in either situation. Since WBC was applied within 1 h post-activity in the study by Russell et al. (2017), this could suggest that greater changes in T could be initiated immediately post-rugby competition, especially since McLellan et al. (2010), observed a return to T baselines within 24 h. This potential should be explored further. In addition, it has been observed in rugby players that decreases in T are associated (r = -0.6) with fatigue and, potentially overtrained states (Maso et al., 2004), and hence, as suggested above, the identification of intensified periods of activity during a season may warrant the frequent use of WBC to mediate these periodic states.

Therefore, there is potential to support the second working hypothesis in that different acute responses to WBC were observed when preceded by differing stimuli i.e., training or competitive match activity. However, further clarification of this *via* baseline testing and controlled comparisons is required.

WBC timing, relative effect, and dosage.

A clear suggestion here is that any benefits which can be acutely instigated by WBC are more likely observed post-match activity rather than post-training. It should be noted that WBC was applied immediately following the training session at M1+3, whereas post-match, WBC was applied 12-h later following a sleep period. While this

appears to represent a potentially unfair comparison, there is the notion of the largely differing stimuli between training and matches, and so the logical progression of the findings of this study is to observe the effects of WBC immediately following matches, rather than after 12-h and a sleep period. Douzi et al. (2018 & 2019) have shown benefits of the application of WBC after physical activity and prior to sleep periods in footballers and runners, again, further supporting the need to further investigate the immediate use of WBC post-competition. Given that the immediate response to a highly stressful environment has already passed by a 12-h period including overnight sleep, it therefore raises the question as to whether the application of WBC at this point has missed the window of opportunity for any significant impact upon endocrine regulation.

Finally, the applied dose of WBC was given according to the potential benefits shown by previous studies (such as Wilson et al., 2019). Since no trial has directly compared single versus multiple exposures to WBC, this warrants further investigation in comparison to a controlled trial without WBC influence.

Conclusion

Frequent use of WBC during a post-match recovery may assist in mediating future post-competition stress responses in order to better control levels of cumulative fatigue. However, there is also the potential for attenuating desired anabolic training responses if used during training periods. Effectively, this could create a paradoxical situation where repeated use of WBC is concerned during competitive periods.

Given the findings and limitations discussed in this study, the following should be addressed to more accurately inform the practice of WBC:

1. Quantify the intensity to reflect the level of stress induced by a competitive match.
2. Establish baseline levels of measurement on which to compare.
3. Employ a control situation/trial which does not utilise WBC.
4. Investigate the applied dosage of WBC exposure immediately post-match to attempt to optimise the endocrine response.

Chapter 6

Effects of exposure frequency of whole-body cryotherapy upon endocrine and biochemical fatigue markers following matches in elite rugby league players.

Data from this paper were presented at the following conferences:

Effects of whole-body cryotherapy on post-match recovery in elite rugby league players. Rugby League Practitioners Conference, (2016) Leeds Beckett University.

Effects of whole-body cryotherapy on post-match recovery in elite rugby league players. Rugby League Practitioners Conference, (2017) University of Chester.

Introduction

The systematic review of this thesis highlighted that the influence of whole-body cryotherapy (WBC) upon recovery following a live competitive sport situation has not yet been investigated. Nor have recovery periods beyond 24 h been examined in experiments using WBC to enhance recovery for concurrent changes in a) muscle damage (*via* creatine kinase), and b) hormonal profiles such as cortisol (C) and testosterone (T). Literature regarding recovery in elite participants within a collision sport environment such as in Rugby League (RL) is also scarce (McLellan et al., 2010; 2011a; 2011b), and to the author's knowledge there are no published studies where WBC is considered in this context.

RL is an intermittent, high intensity team collision sport consisting of 13 players per team played over an 80-min match consisting of two 40-min halves (Gabbett, 2005). Players cover distances of between 8 to 10 km during a match, with up to 10% of this consisting of repeated (short distance) high-speed running, accelerating and decelerating (King et al., 2009). These are activities which require intense muscular outputs, particularly involving heavy eccentric loads which instigate local muscular damage and accumulated metabolic and central fatigue during a match. Repeated blunt force trauma occurs as a result of repeated tackling during the game, again, heightening the stress response and recovery demands following a rugby league match (Twist & Sykes, 2011). The overall result is a distinct alteration of homeostatic body status and is suitably reflected by biochemical and endocrinological profiles (Tavares et al., 2017). McLellan et al. (2010) observed that a time period of up to 120 h post rugby match competition may be required to allow the concentration of creatine kinase (CK) to return to pre-match levels, and the balance of salivary hormone concentrations of C and T to normalize during a typical post-match period.

The majority of studies utilising WBC as a recovery modality following physical activity have used a single, three-minute exposure to WBC, whilst only two studies (Wilson et al., 2018; Wilson et al., 2019) have used more than a single exposure of WBC within a designated treatment session, albeit, at higher temperatures (-85 °C) than those typical of WBC (<-110 °C). As such, it is not clear whether repeating the exposure to

WBC is any more beneficial than utilizing a single treatment and therefore warrants investigation.

To the author's knowledge, this is the first study to investigate the effects of WBC on the combined profiling of hormonal and biochemical markers following elite, contact sport in-season competition. The aim of this study was to compare these responses in RL players during a post-match period when the immediate (<1-h) post-match routines contained either no WBC exposure, a single exposure to WBC, or two consecutive exposures to WBC. In light of the evidence reviewed so far in this thesis, and data from chapter 5, a null hypothesis stating that no influence of WBC exposure upon the recovery process was tested.

Method

Participants

Twenty-three elite RL players (mean ± SD: age 28.0 ± 4.9 years; height 1.84 ± 0.10 m; mass 96.0 ± 9.6 kg) volunteered to participate in the study. Informed consent was obtained from all participants. Prior to the study all participants were briefed on the purpose, procedures and benefits of the study. The study was approved by the University of Bolton Research Ethics Committee.

Experimental Approach

Elite RL players from a European Super League team were monitored *via* biochemical and saliva sampling over three competitive evening matches and their subsequent recovery periods in order to observe CK, T and C responses following varied exposure to WBC. Match cycles followed the sample time structure outlined in the schematic in Figure 6.1. The three post-match intervention protocols were referred to as WBC0, WBC1, and WBC2, reflecting the number of WBC exposures involved in the post-match period. The WBC1 and WBC2 trials were integrated into the team's typical recovery schedule as dictated by the club's performance staff, and, therefore, the experimental variable (i.e., WBC) was not randomised in terms of its application to participants.

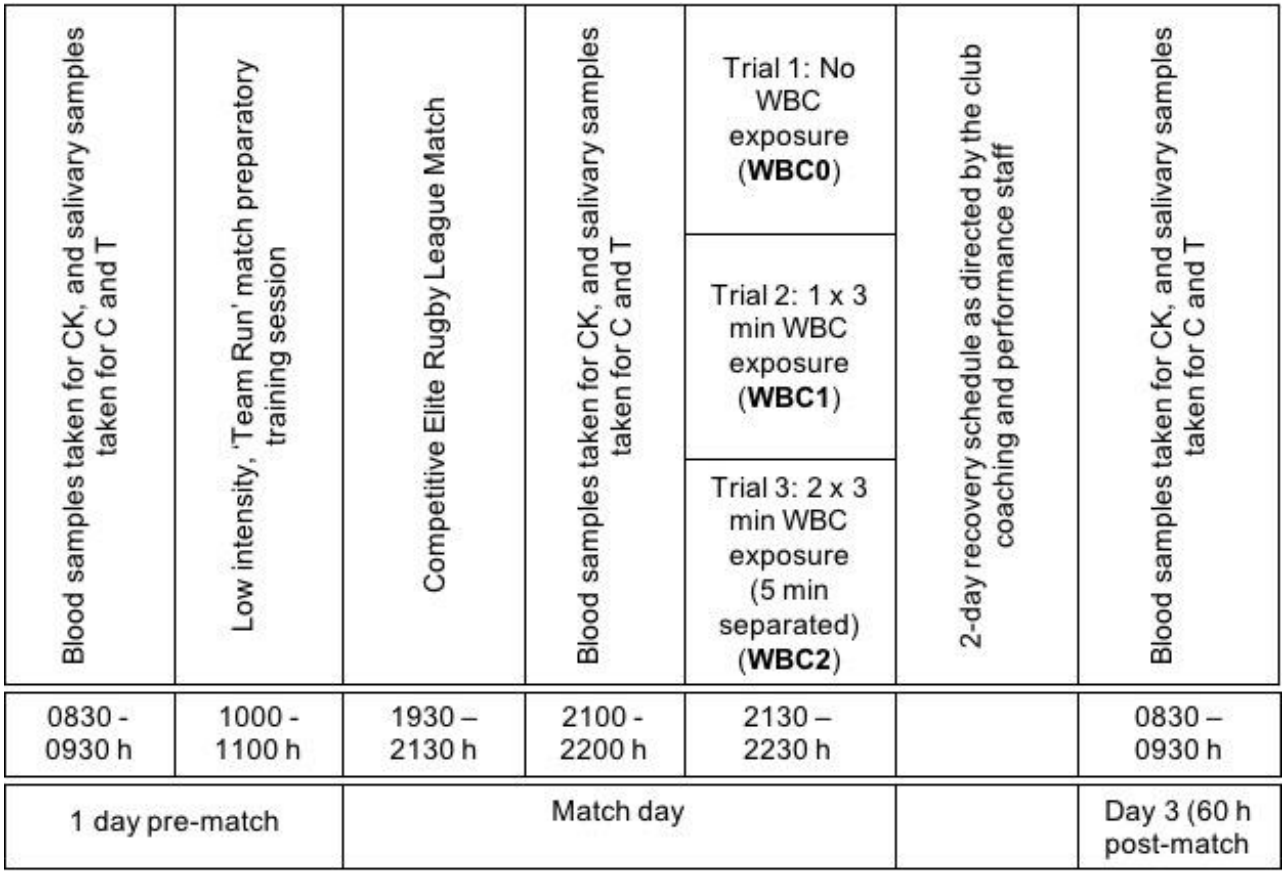

Figure 6.1. Study schematic showing sampling times and experimental trials over a five-day period.

The sample time points used in each individual recovery cycle were used to generate a temporal response for that experimental trial over a 60-h period, including 36 h pre-match (baseline), and immediately (<20 min) post-match measures. For comparative analysis between cycles, a repeated measures approach was adopted, whereby players who were involved in all three cycles were used for analysis. All sampling occurred during April and June during a regular ESL season.

WBC Procedures

A mobile trailer-mounted liquid nitrogen cryochamber (Sappari, UK), owned and operated by British Oxygen Company Linde (BOC), was used for all WBC treatments. All participants consented to the use of the cryochamber according to the guidelines set by BOC and were cleared of any contraindications according to the BOC mandate by the club doctor. Participants donned the appropriate safety attire consisting of shorts, thick socks, sandals, hat, gloves, elbow and knee joint elasticated bandage coverings, and face mask covering the nose and mouth. Within 1 h after match completion, and after post-match sampling, participants entered the cryochamber *via* an initial acclimatisation chamber cooled to $^{-}$60 °C for 30 s. They then entered the main chamber cooled to between $^{-}$120 and $^{-}$135 °C for three minutes. These application parameters are recommended in order to create sufficient thermal gradients following removal of the WBC stimulus (Westerlund et al., 2003).

For the treatment protocol involving two WBC treatments, the participants had five minutes between WBC treatments standing in atmospheric temperatures (15 to 20 °C) outside of the cryotherapy chamber before re-entering.

Saliva sampling and analysis procedures

Saliva samples were collected as per the sampling schematic in Figure 6.1. The participants were asked to refrain from eating or drinking in the hour prior to sample collection in order to ensure regular salivary content and quality. Compliance was checked when participants arrived at the training venue *via* questioning the timing of their last meal or drink. Compliance was shown to be high by the fact that all samples

were able to be collected within a 1 h period upon player arrival, and prior to starting training sessions. On match days, participants continued their typical match preparations in terms of nutrition, mainly comprising of sports recovery/energy drinks ingested during the game so not to impact performance in any way.

For post-match samples, no drinks other than water were ingested until sampling was complete. For all samples taken, water (100 ml, 15 to 20 °C) was provided five to ten minutes prior to sample collection to rinse the mouth. It was only upon rigorous adherence to these steps that a sample was considered suitable, and reliable for the purpose of analysis. A salivette oral swab (Salimetrics LLC, State College, PA, USA) was used to collect unstimulated saliva, placed beneath the tongue for three to four minutes. Salivettes were then placed in individual storage tubes (Salimetrics LLC), immediately placed on ice and frozen within one hour at <-20 °C until required for analysis. Samples were labelled with individual coding so that participant data was anonymised and allowed for a single-blind sample analysis process.

For analysis preparation, samples were defrosted and centrifuged at 5600 rpm for five min. All saliva samples deemed reliable upon collection, and also deemed usable following sample analysis, were included in the statistical data presented. C (µg/dl) and T (pg/ml) were assayed and analysed in duplicate using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics LLC). Microplate analysis was carried out using a microplate reader with Gen-5 software (ELx-800, Biotek Instruments Inc., Winooski, VT, USA). Standard curves were constructed as per the manufacturer's instructions, standards and sample controls (Salimetrics, LLC).

T/C ratio was determined by dividing the concentration of T by the concentration of C. The manufacturer-determined minimum detection limit for T was 1.0 pg/ml (relative 0.0001 µg/dl), with an average intra- and inter-assay coefficient of variance (CV) of 4.1 ± 4.7 % and <12 % respectively as calculated by the study author. The manufacturer-determined minimum detection limit for C was 0.007 µg/dl (relative 70 pg/ml), with average intra- and inter-assay CV of 4.3 ± 4.0 % and <12% respectively. CV was calculated per duplicate sample during microplate analysis and provided by the Gen-5 data analysis output.

Creatine kinase sampling and analysis procedures

Creatine kinase (CK) was measured *via* 30 µl capillarised whole-blood fingertip samples using spring-loaded, disposable single use lancets. Samples were applied to pre-calibrated Reflotron CK analysis test strips and analysed immediately using a Reflotron Plus spectrophotometer (Boehringer Mannheim). Prior to all sample collection periods, Reflotron Precinorm U Quality Control sample tests were administered. Participant samples were collected as per the schematic in Figure 6.1.

Global position system data

GPS measures were taken using portable GPS devices (SPI-Pro; 5 Hz, GPSports, Canberra, Australia) and an inbuilt triaccelerometer (100 Hz) in order to compare the relative match intensity across the three fixtures. Players were accustomed to wearing the GPS devices during training and matches. Each player was pre-fitted with an appropriately sized vest housing the portable GPS unit between the scapulae underneath their team shirt. Players wore the vest and unit for the warm-up and match period. All data were downloaded to a computer using SPI Ezy and analysed using Team AMS software (GPSports, Canberra, Australia). Total distance covered (m) and total impacts over 8Gs of force were extracted for analysis. Reliability of recording high speed running has been shown to be reduced in comparison to lower speeds with 5 Hz units (Johnston et al., 2014). The inbuilt accelerometer allowed for accurate recording of collisions (McLaren et al., 2016).

Statistical Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) for Windows (SPSS, Chicago, IL), IBM version 26. The normality of distribution was checked using the Shapiro-Wilk test. Differences in the raw data between WBC conditions were evaluated using a general linear model (GLM) with repeated measures, within subject factor ‘exposure to WBC’ (three levels) and within subject factor ‘time point of measure (three levels); and interaction. To correct violations of sphericity, the degrees of freedom were corrected using Huynh-Feldt ($\varepsilon > 0.75$) or

Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, as appropriate. Effect sizes (ES) were calculated from the ratio of the mean difference to the pooled standard deviation. The magnitude of ES was classified as trivial (≤0.20), small (>0.20-0.60), moderate (>0.60-1.20), large (>1.20-2.00), and very large (>2.00) based on the guidelines from Batterham and Hopkins (2006). A one-way analysis of variance (ANOVA) for independent groups was used to analyse the differences in match GPS data across conditions since team selection was not fully consistent for each match. Therefore, a group-based analysis was adopted in this instance to verify that the relative intensity over the three matches was similar. All data remained in original scale measures for statistical analyses and presented as mean ± SD unless otherwise stated. A relative percentage change from baseline (36-h pre-match) measures was also calculated. Statistical significance was set at an alpha level of 5% ($P < 0.05$). When the SPSS output demonstrated significance levels of $P = 0.000$, these were corrected to $P < 0.0005$ (Kinnear and Gray, 2006). A statistical trend was identified with $0.1 > P > 0.05$.

Results

A summary of match GPS data is provided in Table 6.1, all salivary and biochemical marker concentrations in Table 6.2, and between-trial effect size (Cohen's d) comparisons at the 60-h time point in Table 6.3.

GPS variability

Global positioning system (GPS) match data for nineteen different players across the three trials were included when, upon data extraction, the GPS unit data showed a clear timeframe of recording across the full match period. Due to a failure of the GPS recording unit during the matches, GPS data from four players were not included in this study. The one-way ANOVA for independent groups revealed no statistically significant difference across all three matches for both, total distance covered or total number of impacts over 8Gs of force ($F_{2, 29} = 0.99$, $P = 0.38$ and $F_{2, 29} = 0.33$, $P = 0.73$ respectively). A Levene's test showed homogeneity of variance across the three matches for both measures ($P = 0.49$, $P = 0.44$, respectively). Overall, it was assumed

that a similar physiological demand was observed across the three matches (Table 6.1).

Table 6.1. Mean ± SD values for GPS data from each match fixture.

Match Cycle	Total distance (m) covered per player	Number of impacts over 8G per player
WBC0	7721 ± 1005	105 ± 57
WBC1	8291 ± 1269	87 ± 41
WBC2	7540 ± 1427	101 ± 54

Table 6.2. Mean ± SD values for hormonal and biochemical markers at all measured time points and conditions.

	36 h pre-match	20 min post-match	60 h post-match
T (pg/ml) (n=11)			
WBC0	50.7 ± 24.9	54.3 ± 19.1	37.5 ± 11.8
WBC1	39.7 ± 11.9	42.4 ± 13.7	54.0 ± 17.4
WBC2	44.1 ± 11.9	49.2 ± 25.3	107.2 ± 27.3
C (µg/dl) (n=11)			
WBC0	0.51 ± 0.29	1.08 ± 0.66	0.43 ± 0.24
WBC1	0.93 ± 0.37	1.44 ± 0.56	0.63 ± 0.26
WBC2	0.52 ± 0.23	0.95 ± 0.57	0.68 ± 0.39
T/C ratio (n=11)			
WBC0	99.4 ± 28.0	61.7 ± 31.2	92.5 ± 37.3
WBC1	47.6 ± 19.3	31.2 ± 10.4	87.8 ± 25.5
WBC2	95.6 ± 30.1	62.4 ± 25.4	179.4 ± 54.1
CK (U/L) (n=6)			
WBC0	328 ± 162	506 ± 204	481 ± 196
WBC1	333 ± 89	425 ± 75	507 ± 180
WBC2	461 ± 133	555 ± 132	382 ± 146

Testosterone responses

For the analysis of T responses, the GLM revealed a significant effect for condition ($F_{2, 20} = 13.1$, $P < 0.0005$). Pairwise comparisons showed that the WBC2 condition was significantly higher than WBC0 (mean difference = 19.3 pg/ml, 95 % CI = 5.6 – 33.0, P = 0.007; ES = 0.63) and WBC1 (21.4 pg/ml, 95 % CI = 10.6 – 32.3, P = 0.001; ES = 0.72). There was no statistical difference between the WBC0 and WBC1 conditions (P = 1.000).

A significant effect for time was also evident ($F_{1.9, 18.9} = 15.4$, $P < 0.0005$). Pairwise comparisons showed that the overall measure of T concentration at 60-h post-match was significantly higher than that at baseline (36 h pre-match, mean difference = 21.4 pg/ml, 95 % CI = 12.7 – 30.1, P < 0.0005; ES = 0.61) and immediately post-match (mean difference = 17.6 pg/ml, 95 % CI = 3.8 – 31.4, P = 0.013; ES = 0.53). There was no statistical difference between the baseline and post-match time point measures for T (P = 1.000).

A significant interaction was found at the 60-h time point ($F_{2.2, 22.1} = 21.9$, $P < 0.0005$, see figure 6.2). From baseline values, increases of 143 % during WBC2 (107.2 pg/ml, 95 % CI = 88.9 – 125.5 pg/ml) and 36% during WBC1 (54.0 pg/ml, 95% CI = 42.4 – 65.7 pg/ml) were observed. A *decrease* by 26 % (37.5 pg/ml, 95 % CI = 29.6 – 45.4 pg/ml) was evident during WBC0 at the 60-h time point.

Cortisol responses

For the analysis of C responses, the GLM revealed a significant effect for condition ($F_{1.3, 13.1} = 4.6$, $P = 0.043$). Pairwise comparisons showed that the WBC1 condition was significantly higher than WBC2 (mean difference = 0.31 µg/dl, 95 % CI = 0.12 – 0.49, P = 0.002: ES = 0.64). There was no statistical difference between the WBC0 and WBC1 (P = 0.182) and WBC0 and WBC2 conditions (P = 1.000).

A significant effect for time was also evident ($F_{1.2, 11.9} = 21.2$, $P < 0.0005$). Pairwise comparisons showed that the overall measure of C concentration immediately post-match was significantly higher than that at baseline (36 h pre-match, mean difference

= 0.51 µg/dl, 95 % CI = 0.20 – 0.81, P = 0.002; ES = 0.45) and at 60 h (mean difference = 0.56 µg/dl, 95 % CI = 0.22 – 0.89, P = 0.002; ES = 0.52). There was no statistical difference between the baseline and post-match time point measures for T (P = 1.000). There was no significant interaction effect shown (P = 0.091, see Figure 6.3).

Testosterone/Cortisol (T/C) ratio responses

For the analysis of T/C ratio responses, the GLM revealed a significant effect for condition ($F_{1.9, 18.5}$ = 24.7, P < 0.0005). Pairwise comparisons showed that the WBC2 condition was significantly higher than WBC0 (mean difference = 27.9, 95 % CI = 0.6 – 55.3, P = 0.045; ES = 0.53) and WBC1 (56.9, 95 % CI = 32.5 – 81.4, P < 0.0005; ES = 1.01). WBC0 was significantly higher than WBC1 (Mean difference = 29.0, 95 % CI = 12.2 – 45.8, P = 0.002: ES = 0.81).

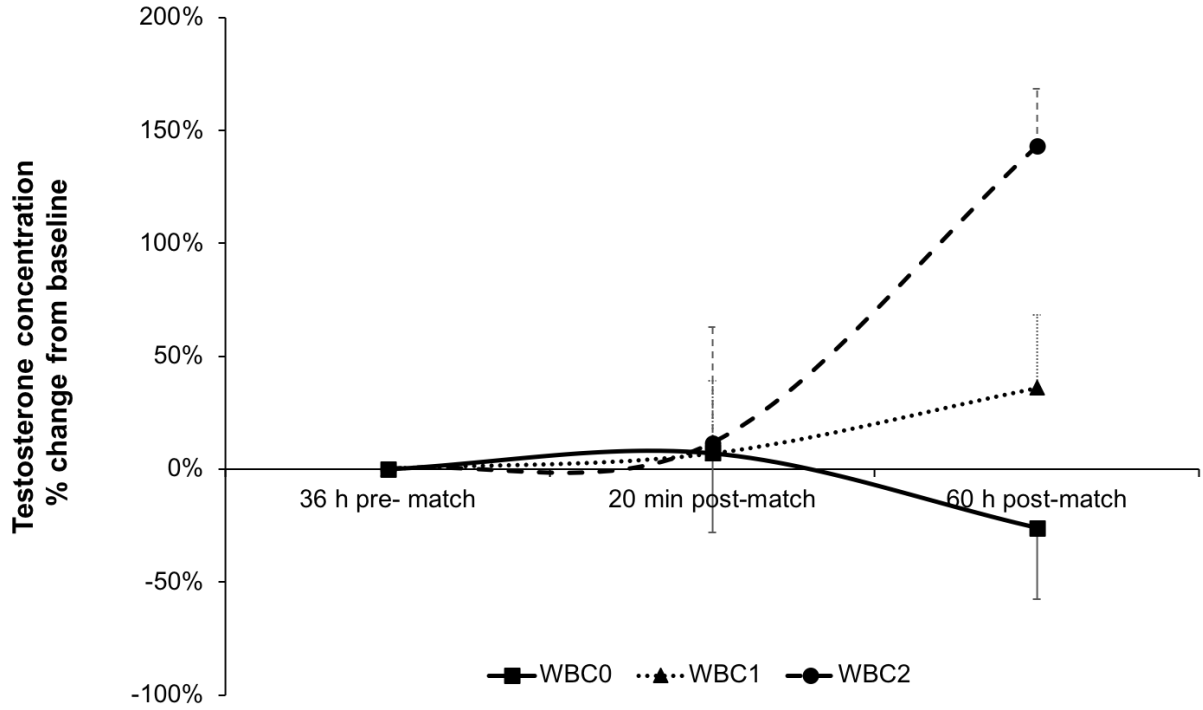

Figure 6.2. Percentage changes of testosterone values from 36 h pre-match baseline for all trials.

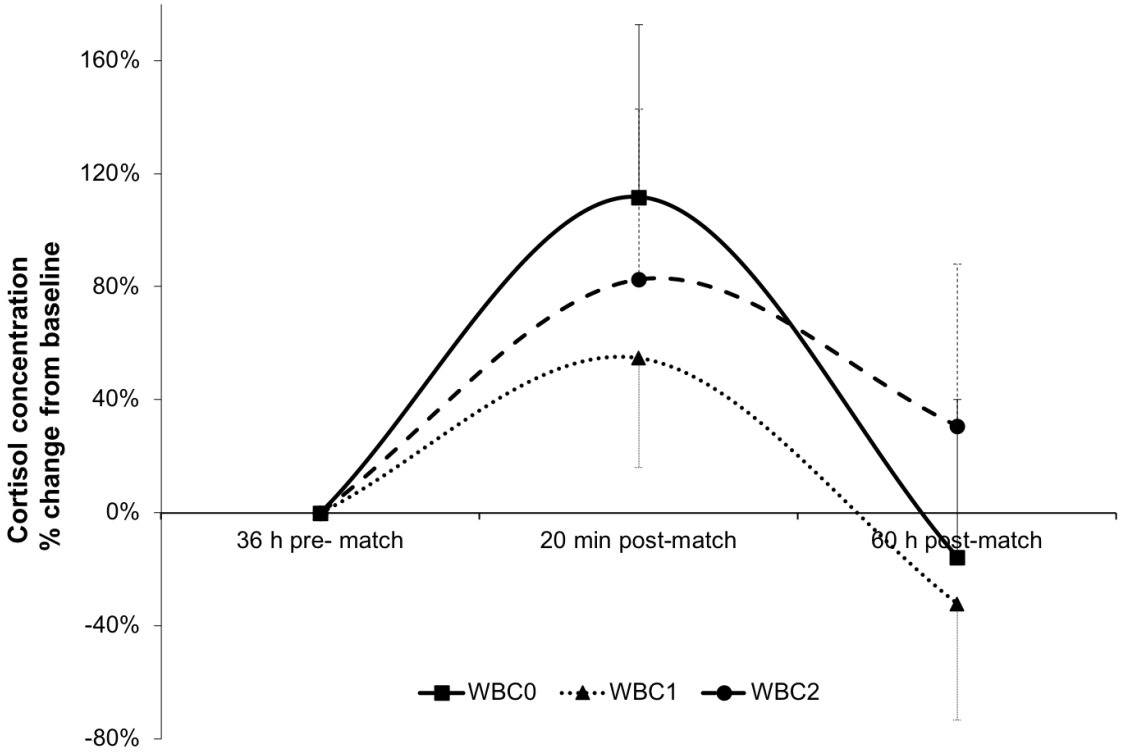

Figure 6.3. Percentage changes of cortisol values from 36 h pre-match baseline for all trials.

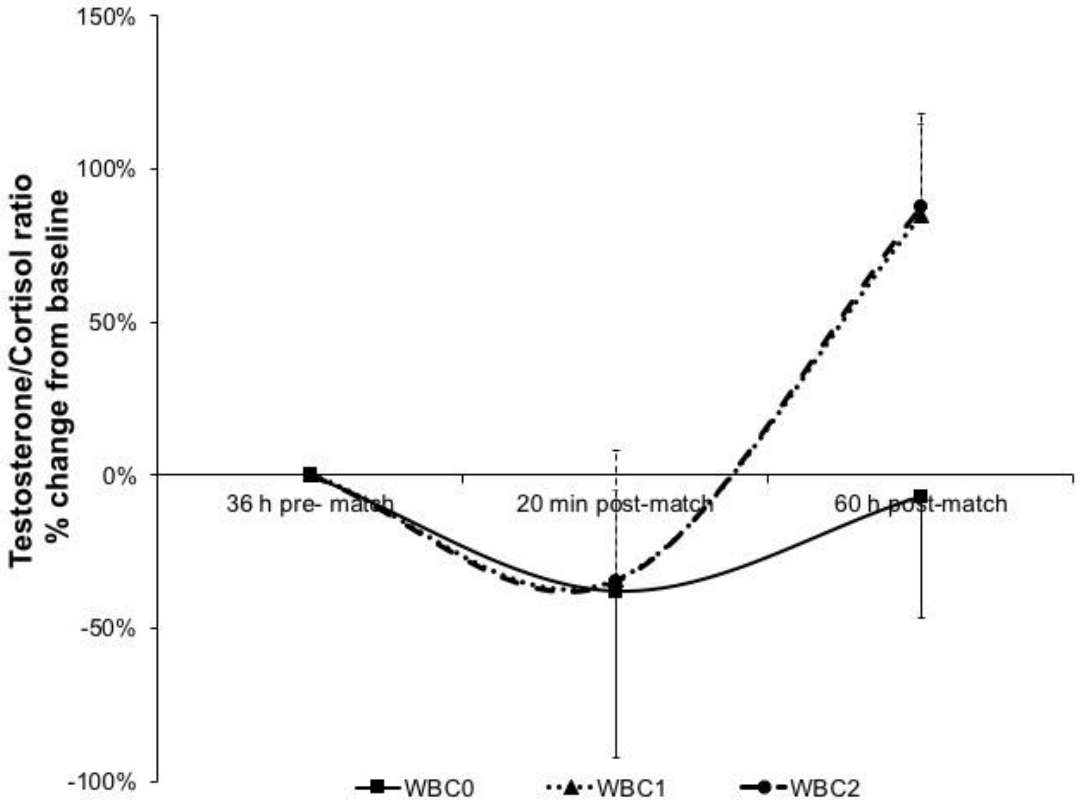

Figure 6.4. Percentage changes of T/C ratio values from 36 h pre-match baseline for all trials.

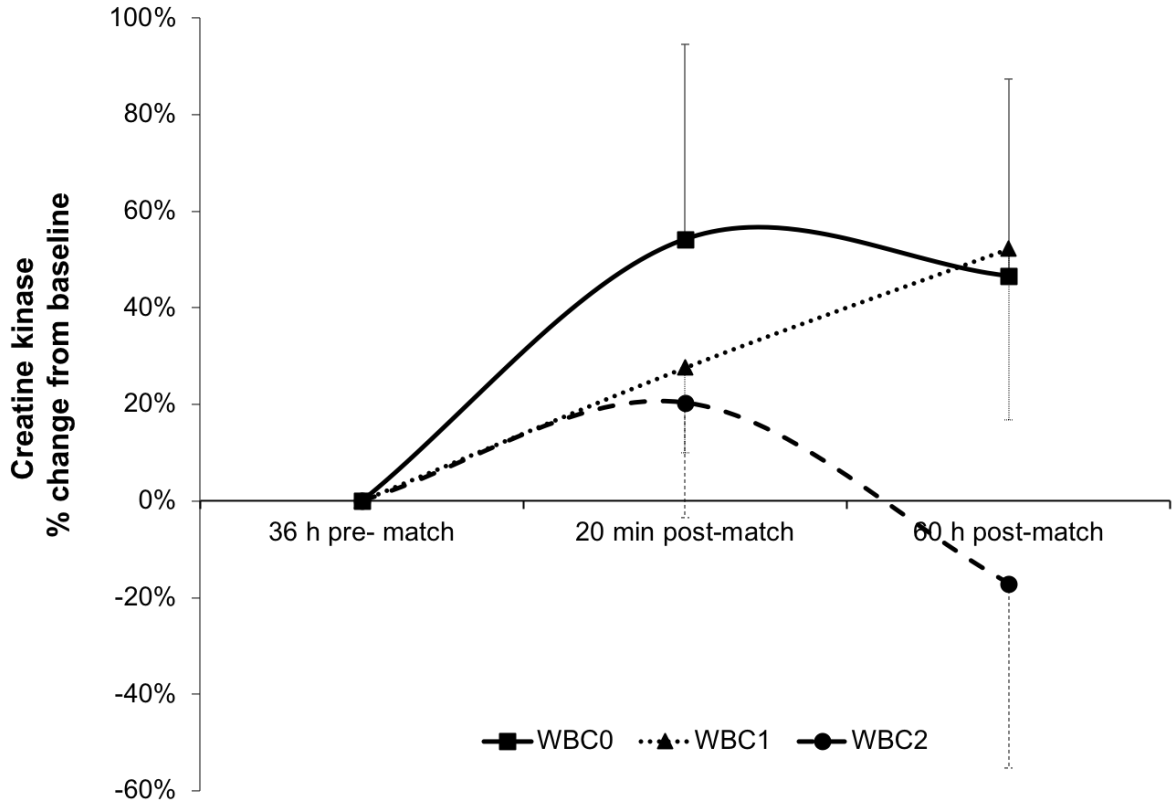

Figure 6.5. Percentage changes of creatine kinase values from 36 h pre-match baseline for all trials.

A significant effect for time was also evident ($F_{1.4, 13.7} = 59.9$, $P < 0.0005$). Pairwise comparisons showed that the overall measure of the T/C ratio immediately post-match significantly decreased compared to baseline values (36 h pre-match, mean difference = 29.1, 95 % CI = 17.7 – 40.4, $P < 0.0005$: ES = 0.21). The relationship value at 60-h post-match was significantly higher than that at baseline (mean difference = 39.1, 95 % CI = 20.9 – 57.3, $P < 0.0005$: ES = 0.45) and immediately post-match (mean difference = 68.1, 95 % CI = 45.7 – 90.6, $P < 0.0005$; ES = 0.54). This observation supports a more catabolic status being reached immediately post-match in all conditions compared to baseline and 60-h timepoints.

A significant interaction was found at the 60-h time point ($F_{2.7, 26.9} = 9.0$, $P < 0.0005$, see Figure 6.4). From baseline values, this equates to an increase of 88% during WBC2 (179.4, 95 % CI = 143.1 – 215.8), 85 % during WBC1 baseline (87.5, 95 % CI = 70.7 – 104.9), and a *decrease* by 7 % (92.5, 95 % CI = 80.5 – 118.2).

Creatine Kinase

For the analysis of creatine kinase (CK) responses, the two-way ANOVA revealed a lack of significance for condition ($F_{1.5, 7.5} = 1.18$, $P = 0.340$) and time ($F_{1.4, 7.2} = 3.2$, $P = 0.109$). The interaction effect showed a statistical trend ($F_{2.0, 10.0} = 3.24$, $P = 0.082$). Figure 6.5 illustrates the CK responses across the three trials. The potential for an interaction effect is more evident when a difference form baseline values is expressed. At 60 h, the CK concentration during WBC2 was 17% *below* the baseline concentration 36 h pre-match. In comparison, the percentage differences during WBC0 and WBC1 at 60 h were 47 % and 52 % *above* baselines, respectively.

Table 6.3. Effect size (Cohen's d) of observed differences between trials at 60 h post-match.

Comparison	T	T/C	CK
60 h WBC2 *vs* WBC1	2.32	2.01	0.76
60 h WBC2 *vs* WBC0	3.31	1.66	0.57
60 h WBC1 *vs* WBC0	1.11	0.23	0.14

Effect sizes (ES) represent: trivial (≤0.20), small (>0.20-0.60), moderate (>0.60-1.20), large (>1.20-2.00), and very large (>2.00). ES was calculated using original values.

Discussion

The main finding of this study was that exposure to WBC within 1 h of match completion irrespective of one or two doses (WBC1 and WBC2), resulted in an 85-90 % elevation relative to baseline in T/C ratio by 60 h post-match. This represents an anabolic hormonal profile which did not appear in the WBC0 trial. Regeneration of damaged tissue is driven by an anabolic environment and so therefore the earlier that this is achieved during the recovery period, the greater the potential for more productive training sessions and future match preparation. It has previously been shown that changes from more relative catabolic states to anabolic states during a post-match recovery period (without WBC influence) are driven through greater changes in C rather than T (Cunniffe et al. 2010; McLellan et al., 2010). Hayes et al. (2012) showed that normal daily hormonal rhythms also display this pattern. However, in the present study, moderate to very large effect sizes (ranging from d = 1.11 to d =

3.31) in T response was observed during the WBC2 trial in comparison the WBC0 and WBC1 trials, whilst C responses were similar across all three trials (Figure 6.3). Only Russell et al. (2017) has previously shown increases in T post-high intensity activity in academy level footballers, however, this data is only evident up to 24 h post-repeated sprint activity. Grasso et al. (2014) noted a significant increase in T concentration following 14 sessions of WBC over a seven-day period during a rugby union international team training camp. Unfortunately, the data from Grasso et al. (2014) is limited to analyses taken at two time points (day one and seven) following two daily sessions of WBC. Nevertheless, the significant increase in T concentration following exposure to WBC in all three studies (inclusive of the present study) shows a promising stimulus of endocrinological function. The mechanism by which this has occurred, unfortunately still cannot be explained by the current data, but the assumption that a stimulation of the hypothalamic-pituitary-gonadal (HPG) axis has occurred certainly warrants further investigation.

Relative to baseline values, and as expected, a catabolic state immediately post-match was observed in all three trials. During the WBC0 trial, an anabolic profile was not reached at 60 h post-match (Figure 6.4). Similarly, the data from McLellan et al. (2010) showed a T/C relationship that barely reached baseline levels up to 120 h post-match, implying that a catabolic state was being maintained during this time. A persistent catabolic state should be considered detrimental for RL players especially during short time periods between matches.

The C concentrations throughout each experimental cycle showed similar responses following rugby league competition. Therefore, it can be assumed that the HPA stimulation as a result of the intensity of each match was similar despite a lack of counterbalancing of trials. This acute C elevation is an important response in responding to high level psychobiological demands (Hayes et al., 2010; Twist and Sykes, 2011). A similar pattern of C normalisation was also observed by McLellan et al. (2011b) where a return to baseline levels was achieved between 24 and 48 h. The consistency of C concentration responses in all three trials in this study may suggest that WBC has no short-term effect upon the acute stress response *via* the HPA axis following high intensity match play involving muscle damage and collisions.

Whilst unable to establish a statistically significant analysis, there are indications in Figure 6.5 that WBC2 influenced the CK appearance in the blood, but reflecting small to moderate effect sizes (d = 0.57 – 0.76). However, it would be inaccurate to assume that WBC concurrently influences a blood marker of muscle damage with the same sensitivity or mechanism as salivary hormone concentration since the characteristics of appearance will vary across the medium used. Furthermore, given that hormonal concentrations are centrally mediated via hypothalamic axes, and CK concentration is an indirect peripheral cell damage marker, it would prudent to assess the effects of WBC upon salivary and blood markers separately and not directly in association.

The data from Table 6.2 shows that the total distance (P = 0.16) and number of impacts (P = 0.65) per player recorded at over 8Gs of force did vary across the three matches, although not reaching statistical significance. Given the large variability of the number of impacts across player position (range 38 – 192 impacts) during a game, some matches could be deemed physically less intense for some players than others. The unpredictability of the live situation makes the direct comparison across matches very difficult even on an individual level. This observation does appear consistent with previous studies which found inconclusive evidence to support the benefits of WBC in assessing peripheral muscle damage *via* CK for a group of athletes.

In terms of endocrine function, the findings do suggest that two, three-minute exposures to WBC immediately following high stress activity have a significant and lasting impact upon hypothalamic balance of HPA and HPG axes during a 60-h recovery period.

Limitations

It should be noted that in aiming to provide a ‘real world’ applied context in RL, the participants acted as their own controls, and therefore in order to maintain a repeated measures approach, participant numbers were difficult to maximise due to team selection, injury, and absence. As such, the comparisons across and within trials became limited to 6-11 participants. In addition to this, it was assumed that the team followed an otherwise typical recovery schedule following the matches featured across the three different sampling trial periods. This included a variety of active recovery

exercise, soft tissue massage and flexibility work. In light of the study, every effort was made by the team staff to replicate the recovery routine where possible, whilst being dictated by the busy season schedule.

Consideration should also be given to the scheduling of sample collection. The circadian rhythm of C and T is such that there is a potential for large variation between morning and evening samples (Hayes et al., 2010). C and T concentrations peak early in the morning (06:00 h), and are at their lowest at 23:00 h, with C demonstrating larger variation (up to 92%) compared to T (42%). In this study, samples were taken between 08:30 and 09:30 h during all sample days, except match day, where samples were taken at approximately 20:00 to 22:00 h. As a result, this may cause some difficulty in analysing the complete effect of WBC, as we are also only providing daily 'snapshots' of endocrine variation that may not fully represent the overriding response (Cormack et al., 2008). Future studies should aim to establish a more periodic reflection of RL player endocrine patterns so that match play responses can be directly compared as opposed to estimated, especially when modalities such as WBC are applied. A logical research development would be to profile the T/C ratio of the athlete longitudinally across a time period encompassing morning and evening measures, and regular seasonal activities in order to establish an individual's baseline 'norm'. In this study, broad between-participant variability was expressed *via* large standard deviations, furthering the need for knowledge of 'true' individual baselines and employing a standardised statistical approach.

Conclusion

This is the first ever study to investigate a dose effect of WBC upon the extended recovery period from elite, in-season collision sport match play in a repeated trial design incorporating control conditions. This study has observed statistically significant and large to very large effect size differences in T following two exposures of WBC for three minutes, administered immediately after competitive play in the real-world environment. This increase was also the main influence supporting a high relative anabolic hormonal balance within 60 h of a competitive match (in comparison to pre-match baselines), which may benefit tissue regeneration and optimise recovery between match cycles. Less conclusive effects of a single dose of WBC immediately

following a match were observed and so practitioners, coaches and athletes should take note of treatment dosage in order to optimise the desired response.

Chapter 7

Synthesis of Findings

The overall goals of this thesis were to investigate the perceptions and current practice *vs* the evidence-based effects of WBC upon fatigue and recovery in elite rugby players.

To surmise, the **aims of this thesis** are as follows:

1. To systematically analyse the current body of research with respect to the effectiveness of WBC upon recovery status following intense physical activities.

This systematic review has highlighted that despite the growing number of studies using extreme cold methods as a stimulus for recovery from exercise, there remains difficulties in the interpretation of findings. This seems due to the inconsistency of methods used in generating a fatigue state. Therefore, at present, it remains difficult to clearly elucidate the real benefits to post-exercise recovery or mechanism of influence of WBC or PBC on an inflammatory, neuromuscular or biochemical level. Where objective markers of enhanced recovery were observed, methodological inconsistencies across studies prevent these mechanisms being demonstrated. Furthermore, this is notwithstanding a large potential for placebo-driven changes given the unavoidable bias of non-blinding or modality concealment to extreme cold exposure. The clearest conclusion being that there appears to be more of a symptomatic or perceptual benefit to recovery using WBC, lesser so in the case of PBC. The possible mechanisms may be due to centralized and basal level responses involving stimulation of parasympathetic responses, improved sleep benefits which may be the underlying driver for recovery from activity.

2. To investigate the current perceptions, beliefs and practices of WBC within elite collision sport team settings in order to analyse its current use.

An online survey gathered information from rugby league, union, and football populations; the findings of which may help inform the understanding of athletes' fatigue states and recovery following training and matches. Firstly, practitioners may not accurately perceive the extent and nature of the fatigued state of athletes. Therefore, accurate and agreeable methods of monitoring should look to draw viewpoints together using subjective and objective means. Recovery is challenged by

evening activity more so than that in the morning or afternoon. Particularly from matches, which appears to be driven by poorer sleep quality and less time to recover. In regard to extreme cold therapy, the adoption of WBC practice is low in this particular group, and implementation methods are variable and generally uniformed. However, the effects of WBC are perceived to be very positive, and the potential adoption for WBC is high, particularly if cost and accessibility barriers to WBC were reduced. This study identified a strong belief that WBC can benefit recovery, mainly by a perceived positive influence on sleep. However, this may only be based upon perceptions from infrequent experiences of WBC, or anecdotal information passed between athletes and/or practitioners.

3. To investigate the effects of WBC upon fatigue characteristics and recovery status following intense physical activities during a live period of Rugby League training and competition.

Frequent use of WBC during a post-match recovery may assist in mediating future post-competition stress responses in order to better control levels of cumulative fatigue. However, there is also the potential for attenuating desired anabolic training responses if used during training periods. Effectively, this could create a paradoxical situation where repeated use of WBC is concerned during competitive periods.

4. To investigate the dose response of WBC and the effects upon recovery following competitive Rugby League matches.

This was the first ever study to investigate a dose effect of WBC upon the extended recovery period from elite, in-season collision sport match play in a repeated trial design incorporating control conditions. This study has observed statistically significant and large to very large effect size differences in T following two exposures of WBC for three minutes, administered immediately after competitive play in the real-world environment. This increase was also the main influence supporting a high relative anabolic hormonal balance within 60 h of a competitive match (in comparison to pre-match baselines), which may benefit tissue regeneration and optimise recovery between match cycles. Less conclusive effects of a single dose of WBC immediately

following a match were observed and so practitioners, coaches and athletes should take note of treatment dosage in order to optimise the desired response.

5. To inform the application of WBC in elite rugby environments with consideration to treatment timing and dosage following RSA using an evidence-based approach.

Two, three-minute exposures of WBC (with a 5-min separation in a normal temperature environment) following high levels of fatiguing activity is recommended such as a competitive match. This should be applied as close as possible following cessation of activity, and before a sleep period to gain greatest benefit. This is emphasised following evening activity when the time available for recovery processes are shortened in light of the approaching sleep period. If activity occurs during other times of the day, recovery appears less challenging due to a wider recovery window and greater time preceding sleep for basal level responses (such as elevated core temperature) to resolve to normal diurnal levels. As such, WBC may not be required in these instances.

General discussion

Strength of WBC evidence

This thesis has clarified that the evidence base for using WBC in a sporting context is small, despite the anecdotal reports of its frequent and high-profile use by athletes. Previously, only one systematic review solely involving WBC and physical exercise had been conducted (Rose et al., 2017). This study established promising outcomes for WBC but only overarching conclusions could be reached given the limited number of similar studies conducted to date. The systematic review of the present thesis has provided the first view of the specific impact of WBC upon post-activity recovery. The inclusion criteria covered participant groups who were physically active, defined protocols of extreme cooling compared with a control trial/group or alternative intervention, extended recovery periods beyond 12 h after activity, and a range of outcome measures to represent the status of recovery. Furthermore, this body of

knowledge was assessed for risk of bias and overall quality using three methods of analysis.

One pertinent finding was that the risk of methodological bias in this particular field is high when WBC trials are compared to controlled situations. Put simply, participants have no means of being concealed or blinded to WBC treatment considering the extreme temperatures involved. Therefore, it is impossible to avoid the potential of placebo in its effects upon an individual. As a result, Chapter 4 partly sought to investigate the beliefs and perceptions of WBC users, and so the potential of placebo-driven effects must be considered and will be discussed later. For future design of WBC studies, concealment of randomised trial order, or group allocation should be maintained as far as possible prior to the application of interventions. Researchers should also use methods of single blinding for analyses. Given the low volume of literature used (15 studies), the combined sample size (n = 244) in the review requires extending, particularly in the elite sporting population in which this thesis aimed to investigate. Only two studies utilised elite-level participants, in particular, trained football players (Russell et al., 2017 and Douzi et al., 2019). Whilst other studies outside of this review have used elite rugby players in their analyses, the specific parameters for post-activity recovery were not met (Grasso et al., 2014; Banfi et al., 2009). As such there is a high need for study designs to address post-activity recovery in the rugby population. Post-activity responses to WBC in female groups also require investigating. In this review, only one study contained a small proportion of female participants and therefore the current transferability of findings are limited predominantly to male populations. For these reasons, the overall strength of literature at this point in time, was judged to be low *via* GRADE analysis.

Pooled data findings of WBC studies

However, the systematic review data analysis did raise some provoking findings when controlled *vs* cryotherapy trials were appropriately pooled. There appears to be a potential for enhanced recovery *via* neuromuscular, perceptual, anti-inflammatory, and endocrinological mechanisms. MVIC and perceived soreness ratings may return to a baseline status (within 5 % or a score of 1/10, respectively) at 72 h post activity, whereas regular (controlled trial) recovery of soreness and MVIC may be extended to,

or beyond 96 h. Lowered CRP and sICAM, and increased IL-10 and testosterone were noted from the pooled data presentation under WBC conditions. These findings lend themselves to suggesting that following intense physical activity, an acute exposure to extreme cold could cause mediation of the inflammatory response, a stimulation of hypothalamic-pituitary interaction, and a heightened cytokine cascade within the initial 24-h period post-activity. As yet, little data exists which expresses the endocrine and inflammatory responses beyond 24 – 48 h in the context studied. Nevertheless, research involving rugby athletes during training blocks concur with the similar effects of WBC on the inflammatory and endocrine markers mentioned above. Unfortunately, experimental conditions in these studies were too variable to meet this review's inclusion criteria (Banfi et al., 2009; Grasso et al., 2014).

In this review, no conclusive differences were noted between PBC (3/5 significantly beneficial studies) and WBC (8/10 studies), however, insufficient data were able to be pooled in order to make clear significant inference. Furthermore, the large heterogeneity of study methods prevented a meta-analysis from being performed. Both WBC and PBC were reported to improve sleep quality, restore MVIC faster, and lower perceived soreness (Tables 3.1 – 3.4), however, the mechanistic markers *via* inflammatory and endocrine function were only investigated in WBC studies. As such, the main driver of the potential mechanisms underpinning post-exercise WBC and PBC are yet to be fully confirmed. Outside of the sporting context, comparative (WBC *vs* PBC) investigations by Hausswirth et al. (2013) and Louis et al. (2015) did find that both modalities demonstrated statistically significant acute effects on the autonomic nervous system and parasympathetic activity. However, greater autonomic responses were noted after using WBC. According to these studies the current reasoning still remains that greater trigeminal stimulation *via* exposing the head to the extreme cold during WBC, instigates a greater stimulus of vagal tone (Louis et al., 2015). Since this review cannot elucidate any specific differences in the post-exercise recovery context, further studies comparing WBC and PBC for recovery purposes in contact sport are needed.

Use, perceptions and beliefs of WBC

Chapter 4 presented the first ever dataset to report athlete and practitioner use, perceptions and beliefs surrounding WBC. Amongst the sample of participants in rugby league, rugby union, and football (n = 115), it was established that the adoption of WBC was low and infrequent, and implementation methods varied. This not only suggests that current practice requires a stronger evidence base on which to establish optimal practice, but also more substantial evidence to convince potential users that the benefits outweigh the issues surrounding cost and accessibility. These particular issues were drawn from the small number of negative responses regarding WBC as to why one would opt to not utilise WBC. Further, minimal responses (n = 2) showed a lack of belief in the potential benefits of WBC in this particular sample. In light of the extensive positive perceptions and beliefs in WBC as shown in Chapter 4, three key findings from Chapter 3 are supported. Firstly, the symptomatic benefits of recovery (a reduction in soreness) appear consistent with athlete perceptions in that 98% of respondents who had used WBC believed that it reduced muscle soreness. Secondly, the potential for placebo effects cannot be discounted where a strong belief in its effects are present even from those who had not experienced WBC. The notion of positive influences to the 'Bayesian Brain' may create top-down, or neurologically encoded predictions about the sensations brought about by the extreme cold. Hence any true mechanistic effect will be interpreted alongside beliefs and perceptions within the nervous system in predicting the body's health status (Ongaro & Kaptchuk, 2019). This view will create challenges for future research studies, and therefore, a form of placebo-control will be necessary in addition to intervention control. So far, only two studies (Wilson et al., 2018; 2019) have implemented some form of placebo control using placebo recovery pills or nutritional interventions. Thirdly, and perhaps most fundamentally, athletes strongly reported greater sleep benefits following WBC, also strongly evidenced by two reviewed studies (Douzi et al., 2018; 2019). The importance of sleep for athlete recovery has been well documented elsewhere, particularly involving high intensity team sport athletes (Davenne, 2009; Venter, 2014; Fullagar et al., 2016; Tavares et al., 2017; Aloulou et al., 2020).

Responses to WBC

The Chapter 4 survey data relating to sleep quality has provoked the need to draw together time of day characteristics, timing of activity, the challenge of recovery, and the effects of WBC. Firstly, it has been evidenced recently that athletes typically struggle to achieve good quality sleep on the night of competition, particularly if the timing of activity occurred after 18:00 h (Roberts et al., 2019). The responses of athletes in the present study support this with 78 % reporting reduced sleep quality and 67 % reporting reduced sleep time post-match. Furthermore, 62 % of athletes reported an increased challenge of recovery from evening fixtures. Biologically, this may be driven by a number of factors immediately post-match. Data from Chapter 6 corroborates other studies *via* an immediate elevation of cortisol following elite rugby league fixtures (McLellan et al., 2011a; Slimani et al., 2018). Veale and Pearce (2009) observed post-match elevations of core temperature to 39 °C in Australian Rules Football Players. Increased sympathetic activity shown by elevated α-amylase is also consistent following competitive physical activity (Kivlighan & Granger, 2006). Collectively, highly stressful competition creates a biological challenge for reversing these responses after matches, more so when the time to sleep is either lessened or delayed (Roberts et al., 2019).

Whilst the qualitative data in Chapter 4 suggests that sleep benefits from WBC are perceptually evident, the mechanisms of this can only be indirectly inferred via previous studies. Any speculation that WBC can influence a resetting of disturbed circadian rhythms of, for example, melatonin remains unconfirmed (Douzi et al., 2018). Allowing for variables such as chronotypes and within-team positions (e.g., backs v forwards) may shed more light in how WBC could create an individualised response. However, the effects of WBC upon the autonomic nervous system appear much more conclusive (Louis et al., 2015; 2020). Louis et al. present strong evidence in both of their studies that stimulation of the autonomic nervous system is dependent upon the extreme cold intensity. An immediate drop in skin temperature stimulates a sudden sympathetic response, peripheral vasoconstriction and subsequent release of norepinephrine. Redirection of blood volume to core organs occurs, which then creates an immediate increase in blood pressure. Compensatory vagal (parasympathetic) activity then ensues due to the need for the reduction of blood

pressure (Louis et al., 2020). From here, the effects of downregulation and preparing for sleep appears enabled in the short term. Data from Chapter 5 show minimal change in the immediate responses of T and C from pre-WBC to immediately post-WBC. As such, any endocrine response must happen secondary to central neurovascular control.

The timing and temperature of WBC exposure also appear crucial in determining delayed responses. In Chapter 6, WBC was implemented within 1 h post-match, delivered between -120 °C and -135 °C, with significant increases in T and the T/C ratio over a 60-h period. Costello et al. (2012) observed no differences in comparison to a control group when WBC was used the following day after fatiguing activity. Kruger et al. (2019) followed a post-activity WBC exposure with another exercise bout before a recovery period ensued and found no differences to a control trial. All other studies reviewed in Chapter 3 employed WBC or PBC within 1 h post-activity and maintained uninterrupted recovery periods. Those studies using temperature ranges from -110 °C and below (all except Wilson et al., 2018; 2019 who used -85 °C) demonstrated beneficial effects of extreme cold exposure post-activity compared to controlled situations. As such, it appears that exposure to WBC (or PBC) should occur in close time proximity to completing the fatiguing activity, and prior to sleeping. The temperature should also be lower than -110 °C. Evidence from Louis et al. (2020) also support the need for extreme temperatures to be at least -110 °C. They noted significant differences in autonomic responses when comparing to -10 °C and -60 °C, however, this was not in a recovery context. In terms of repeated extreme cold exposures in the days following activity, only few controlled studies exist in order to allow analysis of how timing and exposure frequency interact. Ziemann et al. (2014) delayed the initial exposure following activity by 24 h, subsequently observing little difference to controlled conditions until 120 h post-activity. However, Fonda and Sarabon (2013) noted changes between 24 and 72 h when applying extreme cold exposures within 1 h post-activity and then daily until 120 h. As such, these two variables require further investigation.

The specific mechanisms of recovery during subsequent days are still difficult to pinpoint, since, as highlighted above, the responses of recovery markers are highly likely dependent upon the initial stimulus post-activity. Data from Chapter 6 suggest

that one or two 3-min WBC exposures have no acute influence upon cortisol concentration in any situation observed, and therefore the HPA axis appears unaffected. A dose-response effect was present where testosterone concentrations were concerned. A single exposure to WBC was somewhat beneficial compared to controlled recovery at 60 h post-match. However, a second, repeated exposure to WBC within 5 min of the first created a large effect size of change in T and the T/C ratio at 60 h. Louis et al. (2020) present strong evidence that a greater magnitude in autonomic nervous system stimulation could be dependent upon the cold intensity, and therefore an increased temperature after drop is likely initiated by the second WBC stimulus used in Chapter 6. These authors observed an extreme skin temperature reduction of a mean 42 % which coincided with acute catecholamine release after a single session of WBC at -110 °C. A delayed dominance of parasympathetic control followed over a period of 5 days in their study. The reasoning for the acute increase in testosterone in the present study may be as a result of interactions between the ANS and HPG axis, however no previous research can confirm a direct link between increased testosterone stimulation and vagal tone in this context. A recent study by Eda et al. (2020) has shown that a stimulation of parasympathetic activity through 90 min of yoga stretching was accompanied by short term increases in T, the T/C ratio, mood state and feelings of wellbeing. Whilst an athletic population and recovery context was not used, and only short-term responses (<2 h) monitored, an overall optimal balance of basal control systems and perceived wellbeing may well underpin many of the findings shown across this thesis. As ever, more research observing these markers could support the interactive mechanisms of WBC during a post-activity recovery period.

Finally, it appears that following the greatest stimulus of fatigue (i.e., a competitive match), WBC offers a strong influence upon the restoration of hormonal balance, and furthermore offers the potential for earlier, more optimised recovery and a ‘window of opportunity’ for greater training or performance preparation benefits. Data from Chapter 4 showed that, on average, athletes felt that high intensity training sessions could be adequately achieved at 3 d post-match. Given that previous research has shown that endocrine and biochemical markers of recovery do not always resolve within 5 d post-competitive elite level rugby (McLellan et al., 2011a), the high anabolic status shown at 60 h post-match in this study may provide benefits to athletes in the

general coping of physical and psychological pressures of busy training schedules and a readiness for activity (Maso et al., 2004; Crewther et al., 2013). It has already been shown that during a two-week intensified training period, the regular use of WBC helped to avoid symptoms of overtraining and heightened stress (Schaal et al., 2015). When put into context of competitive activity, the proposed benefits of avoiding day-to-day stresses and optimising recovery between matches can greatly benefit athletes (Maso et al., 2004). The use of WBC following training, however, should be used with caution since it has been shown in Chapter 5 that a potential downturn in catabolic hormone balance could result. As already highlighted in this synthesis, time of day of activity and its proximity to sleep periods is an important consideration for recovery. Future research should aim to incorporate these factors carefully in order to investigate effects upon diurnal timing of activity and WBC exposure.

Chapter 8

Conclusions and Recommendations

The overarching goals of this thesis were to investigate the perceptions and current practice *vs* the evidence-based effects of WBC upon fatigue and recovery in elite rugby players. Following a review of related and underpinning literature, this thesis employed a systematic review, qualitative survey study, and two field-based experimental studies in order to synthesise the subjective and objective findings with previous research in this growing body of knowledge. As summarised below, this thesis has been able to draw together findings which are able to (i) support *some* aspects of current practice of WBC in elite sport, and (ii) direct future research in this field.

Practice Recommendations

The first recommendation is to consider the difference in perception between athlete and practitioner when considering fatigue. This study (Chapter 4) has produced evidence that athletes and practitioners may perceive fatigue stresses following training and matches differently. Furthermore, in view of the time-of-day influences upon perceived fatigue and recovery, a more individualised approach may assist the management of player feelings of fatigue. This can therefore influence the recommended use (or non-use) of WBC in the most appropriate situation, whereby a symptom-led approach may instigate the greatest benefits for optimal recovery.

There is likely much greater benefit to using WBC following matches than training since there is a greater stimulus of fatigue. Chapter 5 suggests that the endocrine responses following training are different to those following matches, and the athletes surveyed rated a significantly greater ($P = 0.013$) effect following matches than training.

Some evidence currently exists to suggest that consistent use of WBC can attenuate longer-term (4 weeks) adaptive processes to high intensity interval training (Broatch et al., 2019). So, caution to its regular use (e.g., consistently over full seasons) should be noted until further studies support or refute long term use. Alternatively, previous research does support that where extensive, high intensity blocks of activity are identified (e.g., over a week period), there may be a protective effect of daily use of WBC from symptoms of overtraining (Grasso et al., 2014; Schaal et al., 2015).

Consideration should therefore be given to whether training block goals include physical adaptation over and above homeostatic norms, or the avoidance of long-term fatigue due to a heavy schedule and recovery is the focus. Therefore ‘one-size fits all’ approach towards recovery should not be taken when considering WBC.

Two, three-minute exposures of WBC (with a 5-min separation in a normal temperature environment) following high levels of fatiguing activity is recommended such as after a competitive match. This should be applied as close as possible following cessation of activity, and before a sleep period to gain greatest benefit. This is emphasised following evening activity when the time available for recovery processes are shortened in light of the approaching sleep period. If activity occurs during other times of the day, recovery appears less challenging due to a wider recovery window and greater time preceding sleep for basal level responses (such as elevated core temperature) to resolve to normal diurnal levels. As such, WBC may not be required in these instances.

Accessibility and cost are important considerations. Unless teams have onsite access to a fixed WBC unit, mobile trailer-mounted WBC units are accessible but are costly and may have restricted availability. PBC cryo-stimulation units are more widely available for can be loaned for defined time periods in situ. Some units house only a single person, whereas others may allow up to four per treatment depending on unit size and volume. The current research does support the use of both WBC and PBC delivery apparatus, and effects may be similar. Therefore, teams should plan according to athlete needs and follow a pragmatic approach to when and why it may be used in order to balance the cost *vs* benefit conundrum.

Recommendations for future research

In view of the overall issue with heterogeneity amongst the intricacies of study methods, conducting a meta-analysis would remain inaccurate until a consistent set of studies can be pooled. The key parameter which should receive considerable attention is a consistent fatigue protocol or activity which can impact a multitude of fatigue characteristics. This should combine physical, physiological and cognitive factors which would result in a broad human system fatigue response. The choice of

PBC or WBC should utilise consistent temperature ranges of extreme cold (below -110 °C), and a consistent exposure dosage (2-3 minutes). Chapter 6 in this thesis suggests that two, three-minute exposures of WBC instigate a greater effect upon recovery from competitive activity than a single exposure and should be tested further in controlled studies. A placebo-control and group/treatment concealment to the point of application should also be employed alongside single blind analyses in order to reduce experimental bias as far as is reasonably possible in this context.

The integration of a psychological or subjective measure is vital in addition to selective, centrally derived objective measures such as anabolic/catabolic markers. The concept of the 'Integrative Central Governor' model for the presentation of fatigue suggested by Noakes and colleagues appears pivotal to this point since the fatigue experience is a culmination of biological and psychological phenomena as opposed to purely how quantifiable 'damaged' structures appear to be (St Clair Gibson et al., 2018). Therefore, all future studies with the aim of assessing a whole-body fatigue state should adhere to this point.

Whilst fundamental in biological terms, the endocrinological response is only one component of the recovery process, and so additional mechanisms such as sleep quality, neuromuscular function or subjective ratings have not been explored in this study. Since this is the first study to offer a hormonal profile of recovery influenced by WBC following competitive, high intensity collision sport match play, future studies using participants in RL or other similar level combative team sports such as rugby union or Australian Rules Football should look to replicate and further inform the mechanistic influence offered by WBC. Since there is lack of means to effectively provide a placebo to WBC, perception of its effectiveness and subjective ratings of recovery must be accompanied by physiological markers in order to provide evidence supporting any perceptual benefits.

Finally, the time of day should be considered in the timing of fatiguing activity and WBC exposure. Only four out of fifteen studies reported the time of day in which the protocols occurred and as such no analysis could be performed in this regard within this thesis. In light of the time-of-day physiology reviewed earlier in Chapter 2 of this thesis, coupled with the qualitative findings in Chapter 4 which confirm that timing of

activity is an important factor in the challenge of recovery for athletes, this should be a factor considered in all WBC studies. The evidence reviewed (Douzi et al., 2018 & 2019) and collated in Chapter 4 suggest that the impact upon sleep is a driving factor for recovery processes and well-being, more so when high intensity activity is performed in the evening. A logical next step in study design should be incorporating a two-factor design in which recovery morning or evening fatigue activity receives acute influence from WBC in comparison to controlled conditions. Not only will this explore the time-of-day effects of fatigue activity and WBC, but it will also help to account for diurnal variation in rhythms of core temperature, circulating hormones and/or inflammatory cytokines.

Closing remarks

The overall findings from this thesis support the use of WBC for recovery purposes following intense rugby-specific activity. Qualitative and quantitative findings do draw together the beliefs and perceived benefits of WBC alongside the evidence for its mechanistic impact in assisting player recovery. As such, the future of WBC usage in the contexts of contact sport and practical research studies warrants continuation.

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Appendices

Publication

The following co-authored work has links to the applied field of this thesis in terms of elite rugby league injury and recovery:

Fitzpatrick, A.C., Naylor, A.S., Myler, P., Robertson, C. (2017) A three-year epidemiological prospective cohort study of rugby league match injuries from the European Super League. Journal of Science and Medicine in Sport 21:160-165.

Hopkinson, M., Nicholson, G., Weaving, D., Hendricks, S., Fitzpatrick, A., Naylor, A., Robertson, C., Beggs, C., Jones, B. (2021) Rugby league ball carrier injuries: The relative importance of tackle characteristics during the European Super League. European Journal of Sport Sciences 11:1-10.

Dissemination

Data from this thesis have been presented at the following conferences:

Effects of whole-body cryotherapy on post-match recovery in elite rugby league players. Rugby League Practitioners Conference, (2016) Leeds Beckett University.

Effects of whole-body cryotherapy on post-match recovery in elite rugby league players. Rugby League Practitioners Conference, (2017) University of Chester.

Chapter 4 Questionnaire

Coach, Practitioner and Athlete beliefs, perceptions and practice of cold therapy for recovery in collision sports.

Hello, my name is Adam Naylor and I am conducting a study to investigate the perceptions of fatigue in collision sports and how recovery following training and matches is influenced by cold therapy. This is a questionnaire-based study, which I hope you will be happy to participate in and provide some very useful responses for this area of interest. You would only have to complete this questionnaire once to take part. No further information will be requested from you.

This questionnaire will ask you about:

1. You, your sport and your role in your sport
2. Your perceptions of fatigue and recovery following training and matches
3. Your beliefs and use of cold therapy (such as whole body-cryotherapy, cold compression devices, or ice baths) for recovery

At the moment, little is known about the "hows", "whats" and the "whys" in relation to cold therapy for recovery in collision sports, and so this research seeks to find out more.

By completing and submitting this questionnaire, you are consenting to providing anonymised information for its sole use in this research study. This data may be published in due course to help inform future recovery practices in your sport. Research ethical approval has been provided as per typical university research procedures.

You will not be asked your name or club name. Only your age and sport will be required to categorise information. You may leave or close the questionnaire at any time if you do not wish to complete it.

The questionnaire should take approximately 15 minutes to complete. If you have any queries or wish to contact me regarding this research, feel free to email me at a.naylor@bolton.ac.uk.

Thank you for your valuable time in reading this; I hope that this study can help inform recovery processes with a little more insight to help you in your sport!

Best regards,

Adam Naylor
PhD Student
University of Bolton.

*Required

About you and your sport

This section should only take you 1 minute to complete.

1. 1. What is your age in (full) years? *

2. 2. Are you currently: *

   Mark only one oval.

   - ◯ an athlete?
   - ◯ a coach?
   - ◯ a medical practitioner?
   - ◯ a sport science/strength and conditioning practitioner?

3. 3. What sport do you work in? *

   Mark only one oval.

   - ◯ Rugby Union
   - ◯ Rugby League
   - ◯ Football

4. 4. Typical number of training sessions per week in which you are involved: *

   Mark only one oval.

   - ◯ 10 or more
   - ◯ 7-9
   - ◯ 4-6
   - ◯ 3 or less

5. 5. Typical number of competitive matches per week in which you are involved: *

Mark only one oval.

- ◯ 1
- ◯ 2
- ◯ 3
- ◯ More than 3

Your perceptions of fatigue and recovery following training and matches

This section should take you around 5-6 minutes to complete.

6. 6. Do you / your athletes usually feel fatigued / tired after: *

Mark only one oval per row.

	Yes	No	Sometimes
Matches?	◯	◯	◯
Training?	◯	◯	◯

7. 7. What do you believe to be the main reasons for fatigue after matches (you may tick more than one option)? *

Tick all that apply.

- ☐ Mental tiredness
- ☐ Physical tiredness
- ☐ Damage to the body
- ☐ Other: _____

8. 8. What do you believe to be the main reasons for fatigue after training sessions * (you may tick more than one option)?

Tick all that apply.

- [ ] Mental tiredness
- [ ] Physical tiredness
- [ ] Damage to the body
- [ ] Other: _____

9. 9. (a) What do you believe feeling fatigued after training or matches leads to? (tick one or more option)

Tick all that apply.

	After training sessions	After matches
Muscle soreness	☐	☐
Pain	☐	☐
Reduced wellness	☐	☐
Physical weakness	☐	☐
Reduced sleep quality	☐	☐
Increased sleep quality	☐	☐
Reduced sleep time	☐	☐
Increased sleep time	☐	☐
Increased thinking time when doing tasks	☐	☐
Increased irritability	☐	☐
Increased stress levels	☐	☐
Other	☐	☐

10. 9. (b) If 'other' is stated above, please describe:

_____

11. 10. Do you feel that full recovery after training sessions is normally achieved before the next training session (or match if training the day before a match)? *

Mark only one oval.

- Always
- Frequently
- Sometimes
- Rarely
- Never

12. 11. Do you feel that full recovery after matches is normally achieved before the next match is played? *

Mark only one oval.

- Always
- Frequently
- Sometimes
- Rarely
- Never

13. 12. How long do you believe it normally takes to recover from matches in order to train at high intensity again? *

Mark only one oval.

- 1 day
- 2 days
- 3 days
- 4 days
- 5 days
- 6 days
- 7 days
- more than 7 days

14. 13. (a) Have you used or experienced any of the following ways of monitoring levels of fatigue? *

Mark only one oval per row.

	Yes	No
Self-reporting scales	◯	◯
Physical tests for strength/power	◯	◯
Blood samples for muscle damage	◯	◯
Saliva samples for hormone levels	◯	◯
GPS measurements	◯	◯
Heart rate monitors	◯	◯

15. 13. (b) How useful do you believe these methods are in monitoring levels of fatigue? (Tick one of the four options for each method - scroll across if you cannot see all four answer options) *

Mark only one oval per row.

	Very useful	Quite useful	Not useful	Not sure
Self-reporting scales	◯	◯	◯	◯
Physical tests for strength/power	◯	◯	◯	◯
Blood samples for muscle damage	◯	◯	◯	◯
Saliva samples for hormone levels	◯	◯	◯	◯
GPS measurements	◯	◯	◯	◯
Heart rate monitors	◯	◯	◯	◯

16. 14. (a) Do you think that the feeling of fatigue is greater after: *

Mark only one oval.

- a morning training session?
- an afternoon training session?
- an evening training session?

17. 14. (b) Why do you think this is? *

18. 15. (a) Do you think that recovery is more challenging after: *

Mark only one oval.

- a morning training session?
- an afternoon training session?
- an evening training session?

19. 15. (b) Why do you think this is? *

20. 16. (a) Do you think that fatigue is greater after: *

Mark only one oval.

- a morning match (10:00 h - 12:00 h start)?
- an afternoon match (12:30 h - 16:30 h start)?
- an evening match (17:00 h - 20:30 h start)?

21. 16. (b) Why do you think this is? *

22. 17. (a) Do you think that recovery is more challenging after: *

Mark only one oval.

- a morning match (10am - 12pm start)?
- an afternoon match (12.30pm - 4.30pm start)?
- an evening match (5pm - 8.30pm start)?

23. 17. (b) Why do you think this is? *

Cold therapy for recovery

This section should take you up to 8-10 minutes to complete. The images below show examples of cold therapy techniques you may be familiar with.

Visual examples of Ice baths / cold water immersion (CWI) you may be familiar with. This is very cold water typically between 5 and 15 degrees Celcius. You would normally immerse for between 5 - 15 minutes at any one time. This practice may be repeated.

Visual examples of Whole-body cryotherapy (WBC) you may be familiar with. This is extreme cold air between minus 85 and minus 190 degrees Celcius. You would normally experience this for 2-3 minutes at any one time. This practice may be repeated.

Visual examples of cold compression devices (CCD) you may be familiar with. This is ice and cold water mixed in a device and pumped to a cuff wrapped around a body area. Treatment may last 5 - 20 minutes and may be repeated periodically.

24. 18. (a) Do you currently use, or have previously used, cold therapy devices as part of a recovery routine?

Tick all that apply.

	Whole-body cryotherapy	Cold water immersion / ice baths	Cold compression devices	Other cold method
Yes	☐	☐	☐	☐
No	☐	☐	☐	☐

25. 18. (b) If 'yes' for 'other cold method' is ticked, please state what this is here:

26. 19. (a) What do you believe each cold therapy is designed to achieve? (Tick any which you think apply to each treatment type)

Tick all that apply.

	Whole-body cryotherapy	Cold water immersion	Cold compression devices
Enhance recovery	☐	☐	☐
Reduce muscle soreness	☐	☐	☐
Reduce pain	☐	☐	☐
Speed up healing time	☐	☐	☐
Reduce inflammation	☐	☐	☐
Increased well being	☐	☐	☐
Lower stress levels	☐	☐	☐
Improved sleep	☐	☐	☐
Improved strength levels	☐	☐	☐
Prepare for more training	☐	☐	☐
Not sure	☐	☐	☐
Other	☐	☐	☐

27. 19. (b) If other is selected, please describe. Otherwise, leave blank.

The following questions are addressing whole body cryotherapy (WBC) ONLY. The questions are worded so that athletes AND coaches/practitioners can answer. The 'experience of WBC' refers to athletes, the 'prescription of WBC' refers to practitioners / coaches.

28. 20. Have you experienced (as an athlete) or prescribed (as a practitioner or coach) WBC for recovery? *

Mark only one oval.

- ◯ Yes (Answer the sections in question 21, and complete Q22 if directed by your answers)
- ◯ No (Answer the sections in question 22 ONLY, please leave all sections of Q21 blank)

29. 21. (a) In what circumstances have you experienced or prescribed WBC for recovery?

Mark only one oval.

- ◯ After training sessions
- ◯ After matches
- ◯ Both

30. 21. (b) When was WBC used? (You may tick more than one option)

Tick all that apply.

- ☐ Within 1 hour of match ending
- ☐ Between 1 and 12 hours of match ending
- ☐ More than 12 hours after match ending
- ☐ Within 1 hour of training ending
- ☐ Between 1 and 12 hours of training ending
- ☐ More than 12 hours after training ending

31. 21. (c) Has WBC been adopted into your normal recovery practices after training or matches? (You may tick more than one option)

Tick all that apply.

- [ ] No, it was a one-off use (Please also answer Q22 in addition to Q21)
- [ ] No, it is used infrequently (Please also answer Q22 in addition to Q21)
- [ ] Yes, it is used after most matches
- [ ] Yes, it is used after most training sessions
- [ ] Yes, it is used twice per day (e.g. morning and afternoon)
- [ ] Yes, it is used daily
- [ ] Yes, it is used once or twice weekly
- [ ] Yes, it is used once or twice monthly

32. 21. (d) According to your answer in 21 (c) above, why do you think WBC was used in this way?

_____

_____

_____

_____

_____

33. 21. (e) Have you experienced (as an athlete) or prescribed (as a practitioner or coach) more than one exposure of WBC in a single treatment session? An example of this could be 2 x 3 minutes in the chamber with a time period outside of the chamber in between.

Tick all that apply.

- [ ] Single exposure only (e.g. 1 x 3 minutes)
- [ ] Two exposures in one treatment session (e.g. 2 x 3 minutes)
- [ ] Three exposures in one treatment session (e.g. 3 x 3 minutes)
- [ ] Other: _____

34. 21. (f) According to your answer in 21 (e) above, why do you think this level of exposure was used?

35. 21. (g) How effective do you feel WBC is for recovery after a match?

Mark only one oval.

	1	2	3	4	5	6	7	
Not effective	◯	◯	◯	◯	◯	◯	◯	Highly effective

36. 21. (h) How effective do you feel WBC is for recovery after training?

Mark only one oval.

	1	2	3	4	5	6	7	
Not effective	◯	◯	◯	◯	◯	◯	◯	Highly effective

37. 21. (i) In your opinion, did WBC make recovery:

Mark only one oval.

- ◯ Quicker?
- ◯ Slower?
- ◯ No different?

38. 21. (j) Describe any positive or negative experiences in using WBC as an athlete or observations from prescribing WBC as a practitioner / coach.

Question 22 - Answer this question if you have NOT used or prescribed WBC for recovery, OR have only had a one-off OR infrequent experience with WBC.

39. 22. (a) Given the opportunity, how likely would you regularly use WBC after matches?

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all likely	◯	◯	◯	◯	◯	◯	◯	Highly likely

40. 22. (b) Please provide your reason(s) for your answer above.

41. 22. (c) Given the opportunity, how likely would you regularly use WBC after training given the opportunity?

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all likely	◯	◯	◯	◯	◯	◯	◯	Highly likely

42. 22. (d) Please provide your reason(s) for your answer above.

43. 22. (e) How soon after training or matches would you use WBC?

Mark only one oval.

- ◯ Within 1 hour
- ◯ Between 1 and 12 hours
- ◯ After 12 hours
- ◯ Wouldn't use/advise

44. 22. (f) Please provide your reason(s) for your answer above.

45. 22. (g) How often would you use WBC?

Mark only one oval.

- ◯ Twice daily (e.g. morning and afternoon)
- ◯ Once daily
- ◯ 2-5 times per week
- ◯ Once per week
- ◯ 2-3 times per month
- ◯ Once per month
- ◯ Wouldn't use/advise

46. 22. (h) Please provide your reason(s) for your answer above.

47. 22. (i) How many exposures in the WBC chamber in one single session would you use?

Mark only one oval.

- ◯ Once only (e.g. 1 x 3 minutes)
- ◯ Twice (e.g. 2 x 3 minutes with a short break in between)
- ◯ More than twice
- ◯ Wouldn't use/advise

48. 22. (j) Please provide your reason(s) for your answer above.

49. 23. Is there anything else you would like to add in relation to the main topic of recovery and cold therapy?

Thank you for completing this survey and participating in this research. Your time is very much appreciated. If you do have any questions please do feel free to email me at A.Naylor@bolton.ac.uk.