An Evaluation of the Effects of Whole Body Cryotherapy Treatment for Sports Recovery and Performance

Submitted for the Degree of

Doctor of Philosophy

at the University of Northampton

May 2021

Adnan Haq

Contents

List of Figures

List of Tables

List of Abbreviations

ANOVA – Analysis of Variance

CIVD – Cold-Induced Vasodilation

CK – Creatine Kinase

CMC – Chris Moody Centre

CMJ – Counter Movement Jump

CON – Control

CV – Co-efficient of Variance

CWI – Cold Water Immersion

DOMS – Delayed-Onset Muscle Soreness

EIMD – Exercise-Induced Muscle Damage

EMG - Electromyography

Hb – Haemoglobin

HR - Heart rate

IL-6 – Interleukin-6

ISAK - International Society for the Advancement of Kinanthropometry

MVC – Maximal Voluntary Contraction

PBC – Partial Body Cryotherapy

PGC1α - Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

RBE – Repeated Bout Effect

RPE – Rating of Perceived Exertion

SD – Standard Deviation

sICAM-1 - Soluble Intercellular Adhesion Molecule 1

SPSS - Statistical Package for the Social Sciences

VAS – Visual Analogue Scale

VO_2 max – Maximal Volume of Oxygen Consumption

WBC – Whole Body Cryotherapy

Acknowledgements

A big thank you to my primary supervisors Bill Ribbans and Anthony Baross for their continual faith, guidance and support from the beginning right through to the end of my PhD process. Additionally, the moral support of Wanda McCormick at Moulton College in the first two years of my PhD shall be acknowledged. I am also grateful to the College for the continual funding, as well as assisting with some of the marketing and recruitment.

Completion of a huge project is never possible without the input of support staff. In particular, Chris Talbot, technician at the University of Northampton; and Charlotte Brown of the Chris Moody Centre at Moulton, both key players who have done excellent jobs in accommodating my trials. Other staff members from the college or university that should be thanked include Jason Day, Lee Howarth, Saul Cuttell, Luke Testar, Nick Allen, Valerie Graham, Tony Kay, among others.

In my lively non-academic world, a special thank you to my friends around the globe and my family. Their priceless role for maintaining my sanity shall never be overlooked, more so than ever during the unprecedented Covid-19 pandemic which disrupted my data collection for 7 months, and throughout the wait to be given full lab access post-lockdown.

And last but not least of course, a statistically significant appreciation to the hundred or so participants who gave up their time and effort to perform my data collection trials, enduring varying degrees of exercise related pain in the process. Honourable mentions for participation in more than two studies:

Rob McGoldrick, Russ Dickerson, Adam Hughes, Richard Taylor, Yavor Georgiev and Adrian White.

Also thank you to Mark Kennedy for helping with promoting my studies via Northampton Road Runners as well as taking part in several trials himself.

Muchísimas gracias a todos. Estoy tan agradecido.

Abstract

Whilst Whole Body Cryotherapy (WBC) has become an emerging tool for sport and exercise recovery, its overall efficacy remains contentious. This thesis addressed a variety of issues concerning the practice.

Firstly, the impact of single WBC interventions for treating exercise-induced muscle damage (EIMD) is unclear. Secondly, the influence of inter-individual factors on WBC outcomes post-exercise remains an under-investigated area. Therefore the first main study explored the effects of age and body fat content on responses to WBC following downhill running, a commonly utilised eccentric exercise model for inducing muscle damage. WBC participants underwent cryotherapy (3 minutes, −120°C) one hour post-downhill run and control (CON) participants passively recovered (20°C). Despite the presence of EIMD, WBC significantly blunted ($p=0.04$) the decrease in muscle torque 24 hours after the downhill run. This response was significantly influenced by age, with young participants (<40 years) retaining their muscle strength more than older participants (≥45 years). WBC may therefore attenuate EIMD and benefit muscle strength recovery following eccentrically biased exercise, particularly for young males.

A subsequent downhill run study investigated the influence of WBC timing post-exercise, a factor that could clarify optimal treatment usage. An additional objective was to compare the effects of WBC with cold water immersions (CWI) since the verdict regarding which cold modality is superior for recovery remains an on-going area of controversy. It was revealed that WBC 4 hours post-exercise was ineffective in treating EIMD markers, so applying WBC within one hour after exercise may be preferable to delaying by several hours. However, WBC was no more effective than CWI, meaning that the cost vs. reward implications of WBC treatments would need further reviewing.

Finally, the implications of repetitive WBC during training programmes require further evaluation due to the possibility of repetitive cold interfering with long term adaptations. The final study investigated the impact of two weekly WBC treatments on adaptations to a 6 week strength and endurance training programme. It was found that WBC participants significantly improved their muscle strength comparatively to the CON group. However WBC did not improve their jump height ($p=0.23$) in contrast to the CON group ($p=0.01$). In conclusion, repetitive WBC does not appear to blunt strength training adaptations, although there may be an interference effect in the development of explosive power.

CHAPTER 1 – INTRODUCTION

Cryotherapy is a generic term applied for any cold treatment. Due to its potential in reducing tissue temperature, it has been used for a variety of applications, including alleviating pain, swelling, inflammation, as well as treating musculoskeletal disorders and skin lesions (Lubkowska, 2012). Whole Body Cryotherapy (WBC) is distinguished from other forms of cryotherapy in that the whole body is exposed to extremely cold air (<-100°C), typically sourced from a mixture of liquid nitrogen and air in a closed chamber (Bouzigon *et al.,* 2016). 'Cryosaunas' differ by being an open system where the head is not directly exposed to the cold, thus some articles (e.g. Ferreira-Junior *et al.,* 2014) have referred to this treatment as Partial Body Cryotherapy (PBC). Since the first chamber was built in Japan in 1978, the use of WBC has become more widespread across the globe, raising public awareness. There are now several fixed units scattered across the United Kingdom.

Recent findings have shed light on the potential benefits of WBC on musculoskeletal disorders (Romanowski *et al.,* 2020; Straburzyńska-Lupa *et al.,* 2018) and mental wellbeing (Pawik *et al.,* 2019), whilst its use in sport and exercise recovery has become more documented (Lombardi *et al.,* 2017; Partridge *et al.,* 2019). Sports personnel are familiar with the practice of ice baths or cold water immersions, the use of which has been extensively reviewed (Ihsan *et al.,* 2016; Leeder *et al.,* 2012; Machado *et al.,* 2016). However, the recent emergence of the extreme cold present in WBC chambers has added an additional perspective to sports recovery practice. Several sports have applied WBC in the belief that recovery would be superior to other conventional means and there have been several high profile cases – e.g. Leicester City in their remarkable Premier League winning season of 2015-16. Despite many of the effects of WBC post-exercise (discussed in more depth in chapter 2), the precise impact and efficacy of WBC in this context remains equivocal, with mixed findings.

One significant theme to be explored in this project is exercise-induced muscle damage (EIMD) and how cryotherapy can potentially alleviate its impact. EIMD describes the muscle breakdown and its associated symptoms that typically occur following a range of sports and exercises. For instance, fatigue, swelling and delayed onset of muscle soreness (DOMS), the latter of which typically peaks 24-48 hours after exercise (Fatouros & Jamurtas, 2016; Owens *et al.,* 2018). Whilst the complex mechanisms of EIMD have already been reviewed (Owens *et al.,* 2018; Peake *et al.,*

2017), a consensus has yet to be reached as to what is considered the optimal means of mitigating this damage, therefore supporting athletic recovery.

In the current field concerning sports recovery and performance, there remains a need to address the efficacy of WBC in further depth to better inform the sporting community of its overall merit. This is particularly necessary when its expense is factored in - significantly more than £80,000 for chamber installation alone (Bouzigon *et al.,* 2016), thereby being a far costlier tool than ice packs and cold water baths. The economic implications of the cryotherapy chamber used in this thesis will be reviewed in chapter 8.

This thesis highlights some important grey areas in the study of WBC for post-exercise recovery and sports performance. Specifically, the research aimed to address five objectives:

1) To investigate the effect of WBC for treating EIMD following a specific eccentric exercise protocol (chapter 4).
2) To investigate the influence of individual differences (age and body fat) in response to WBC treatments post-exercise (chapter 4).
3) To determine the influence of treatment protocol factors – i.e. timing (chapter 5).
4) To compare the effectiveness of WBC with cold water immersions post-exercise (chapter 5).
5) To investigate the potential impact of frequent WBC treatments on training adaptations (chapter 6).

These issues are further explored throughout a series of studies covered in the aforementioned chapters. By utilising the Chris Moody Rehabilitation Centre at Moulton and a variety of physiological markers, it is anticipated that the findings from such studies further inform sports practitioners optimum practices for WBC treatment, as well as validating the potential benefits for sports recovery and performance.

CHAPTER 2 – LITERATURE REVIEW

The Effect and Use of Whole Body Cryotherapy Treatment.

How Effective is it for Sports Recovery and Performance?

2.1 Cryotherapy: a method of sports recovery

Athletes place considerable demands on their bodies when undergoing training programmes to facilitate competition performance. Of paramount importance is exercise recovery, the ability to restore the body to the pre-workout state, reducing fatigue and injury risk. Recovery is necessary to restore numerous physiological disturbances. Depending on the nature of the exercise, such disturbances might include glycogen depletion, hyperthermia, acid-base imbalances, disruption of muscle fibres (including Ca^{2+} perturbations) and lactate accumulation (Barnett, 2006).

Advances in sport science research throughout the past few decades are likely to be a contributory factor to the increased input and sophistication of sports recovery practice. Recent sporting achievements could be attributed to such advances in our understanding of sport science and how the human body can recover optimally. Several methods of post-exercise recovery have been adopted, notably hydration, nutrition (e.g. protein supplementation), sleep, massage, foam rolling and compression garments (Halson, 2015). One emerging recovery tool is cryotherapy which involves the use of cold temperatures to reduce swelling and soreness and shall be the focus of this literature review. From an athlete's perspective, it is beneficial to accelerate recovery following strenuous exercise to enable them to perform subsequent quality training sessions, taking full advantage of training stimuli and adaptations to improve their skill and performance (Barnett, 2006).

Cryotherapy utilises low temperature adopting techniques such as local applications of ice, immersion in cold water baths or whole body cryotherapy (WBC). Local ice applications have been shown to reduce acute muscle pain following exercise (Gulick *et al.,* 1996) and increase pain thresholds, possibly as a result of blocking hyperalgesic sensory afferents to the brain, thus consequent reduction of nerve conduction velocity (Algafly & George, 2007). Ice baths and cold water immersions (CWI) are perhaps better known in the context of sports recovery. The documented effects of CWI include reductions in core temperature (Peiffer *et al.,* 2010), skin temperature (Costello *et al.,* 2012), muscle temperature (Ihsan *et al.,* 2014), and alterations in muscle blood flow (Mawhinney *et al.,* 2013). Whole body cryotherapy, first introduced in Japan (Yamaguchi, 1978), involves the exposure to extremely cold dry cryogenic gas, usually liquid nitrogen, in a chamber, typically with temperatures lower than -100°C. WBC can mitigate the symptoms of musculoskeletal conditions,

including ankylosing spondylitis (Stanek *et al.,* 2005), adhesive capsulitis (Ma *et al.,* 2013) and fibromyalgia (Bettoni *et al.,* 2013). The majority of clinical benefits reported in such studies are associated with the anti-inflammatory effect of cryotherapy as well as its pain reduction properties. These are also potential mechanisms by which sports recovery can be enhanced (Bouzigon *et al.,* 2016). The effectiveness of cryotherapy from a therapeutic perspective is arguably less controversial than in the context of sports recovery.

One underlying principle of applying cryotherapy is as a treatment for exercise-induced muscle damage (EIMD), a common occurrence following strenuous exercise, particularly if unaccustomed or involving predominantly eccentric contractions (Clarkson & Hubal, 2002). Eccentric contractions involve lengthening of the muscle-tendon complex, causing a larger amount of force generated per muscle fibre (Hody *et al.,* 2019). Characteristics of EIMD include the overstretching of muscle fibre sarcomeres, Z-line streaming, sarcolemmal disruption, loss of calcium homeostasis and a pronounced inflammatory response involving the infiltration of leukocytes (e.g. neutrophils and subsequently macrophages) into muscle tissue with consequent production of cytokines (Peake *et al*., 2017), as well as metabolic disturbances (Tee *et al.,* 2007). Such mechanisms contribute in varying extents to the noticeable reduction in muscle torque generation and the occurrence of delayed onset of muscle soreness (DOMS) (Peake *et al.,* 2017). It is conceivable that the greater the alleviation of EIMD characteristics post-exercise and the quicker the restoration of muscle contractile features (e.g. membrane integrity and excitation-contraction coupling efficiency), the more potential benefits posed to athletes for subsequent exercise bouts.

The overall usage of whole body cryotherapy is not as prevalent as other methods of exercise recovery, partly because of cost and the relative lack of chambers in the UK. However, the use of mobile units and cryosaunas, (suitable for individual use) has made the treatment more accessible in the past decade (Bleakley *et al.,* 2014). Even though there are contraindications for the use of WBC, for instance pre-existing cardiac disease and hypertension (Lubkowska, 2012), the treatment is generally safe with very little risk of frostbite since all extremities are covered and cold application seldom lasts longer than 4 minutes. Participants are generally exposed to cold air in two parts: a vestibule chamber in temperatures of between -40°C to -60°C for a short

period of less than a minute, followed by the main chamber where temperatures range from -100°C to -140°C (Selfe *et al.,* 2014; Wozniak *et al.,* 2007).

Figure 2.1.1: An example of a whole body cryogenic chamber treatment (Chris Moody Rehabilitation Centre, Moulton, Northamptonshire), typically utilising liquid nitrogen as the coolant, with temperatures set at -110°C to -140°C

2.2 Physiological effects of whole body cryotherapy

There are numerous reported effects of WBC treatment on the human body, many of which are summarised in Figure 2.2.1 and reviewed in the sub-sections that follow.

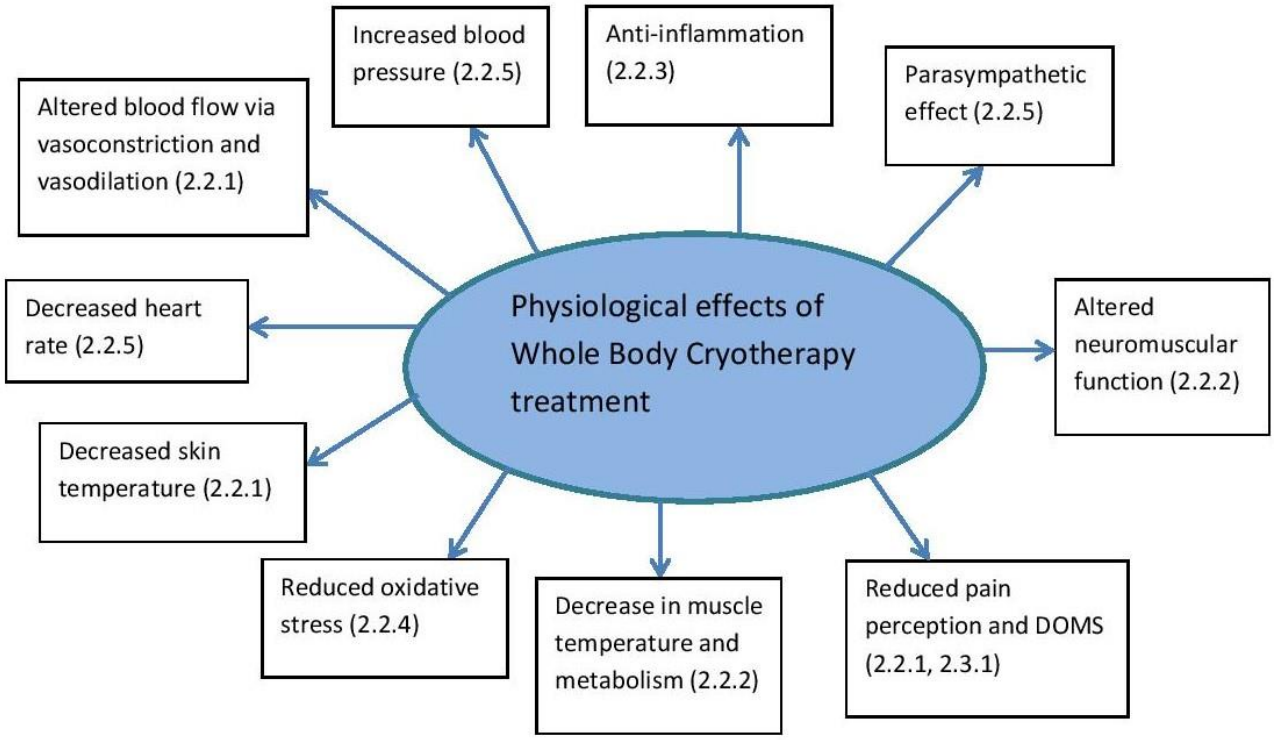


Figure 2.2.1: Summary of some possible physiological effects of Whole Body Cryotherapy treatment.

2.2.1. Skin Temperatures and Blood Flow

One of the noticeable effects of cryotherapy treatment is the impact on skin temperatures and blood flow, which give an indication of heat exchange between the internal tissues and external environment (White & Wells, 2013). Using infrared thermal imaging techniques on regions of the lower limbs, researchers have reported significant reductions in skin temperature following WBC. For example, an average skin temperature drop of 13.7°C was observed following 3 minute WBC exposures at -110°C (Hausswirth *et al.,* 2013). This could have implications for pain perception, since it has been reported that a skin temperature reduction to a 13.6°C threshold is required to obtain an analgesic effect (Westerlund *et al.,* 2009). Several studies (Bailey *et al.,* 2007; Hausswirth *et al.,* 2011) have demonstrated that cold applied after exercise training can increase pain threshold.

Additionally, Costello *et al.* (2012) reported significant reductions in thigh skin temperatures immediately after WBC. However, this decrease in skin temperature was followed by a rapid recovery towards baseline levels within 60 minutes post-treatment (Costello *et al.,* 2012). This skin temperature 'U' response can be attributed to the 'rebound' effect, where there is initially immediate cold induced vasoconstriction, followed by vasodilation with consequent increases in cutaneous blood flow (Lubkowska, 2012). During extreme cold exposure, the body's natural thermoregulatory response is to attempt to retain core temperature and prevent heat loss to the surrounding environment, resulting in the immediate constriction of peripheral blood vessels. Blood is redistributed from the muscles to subcutaneous tissue once the individual is removed from cold exposure (White & Wells, 2013). This hyperaemic effect, or 'cold-induced vasodilation' (CIVD), is theorised to promote recovery through the removal of waste products such as hydrogen ions and inorganic phosphates, as well as enhancing delivery of oxygen to hypoxic tissues (White & Wells, 2013). CIVD can also directly cause reductions in muscle metabolism, owing to the established effect on reduced blood flow to muscles in favour of subcutaneous tissue (Gregson *et al.,* 2011) explained further in section 2.2.2.

2.2.2. Muscle Metabolism and Function

Another consequence of the vascular rebound and CIVD is a gradual reduction in muscle temperature as heat is being transferred peripherally from deep tissues (White & Wells, 2013). Indeed, achieving a notable decline in muscle tissue temperature is a potential rationale for cryotherapy applications with probable reductions in muscle tissue metabolic activity (Bleakley & Hopkins, 2010). A reduced muscle metabolic demand can curtail further muscular damage mediated by reactive oxygen species (described in section 2.2.4) and is associated with reductions in muscle swelling (White & Wells, 2013). Using near infra-red spectroscopy (NIRS) to measure muscle oxygenation, Ihsan *et al.* (2013) were the first to demonstrate *in vivo* that post-exercise cooling can blunt muscle metabolism when applying cold water immersion as a recovery modality. NIRS measurements were subsequently used to demonstrate that WBC can also blunt muscle metabolism (Hohenauer *et al.,* 2020; Selfe *et al*., 2014). However, it should be noted that NIRS does not permit direct measurement of muscle 'metabolism', rather only muscle oxygenation. A reduction in muscle metabolism could mean that surrounding tissues are more tolerant of an ischaemic environment, thus reducing the probability of secondary cell injury (Bleakley & Davison, 2010). The latter concept describes the damage that occurs following immediate muscle trauma and rapid leukocyte infiltration. Whilst it has been shown that a single WBC treatment can induce slight reductions in deep muscle temperatures (Costello *et al.,* 2012; Mawhinney *et al.,* 2017), there is generally a lack of evidence to suggest substantial reductions in deep muscle tissue temperatures post-WBC treatment following exercise. This makes it difficult to conclude the relevance of this physiological response in the context of enhancing sports recovery. In addition, it is difficult to judge the optimal reduction in tissue temperature for sports recovery or clinical effectiveness (Bleakley & Hopkins, 2010).

It is considered that the most reliable marker of muscle function is muscle torque generation (Warren *et al.,* 1999), typically assessed by maximal voluntary contractions using a dynamometer. WBC treatments have been shown to enhance recovery of maximal voluntary contraction torque and hence muscle function (Fonda & Sarabon, 2013; Hausswirth *et al.,* 2011,) an hour following muscle damaging exercise. Additionally, Ferreira-Junior *et al*., (2015) discovered significant benefits in muscle torque for up to 4 days following a single treatment of partial body cryotherapy at -110°C, whereby the participants head is not directly exposed to cold

(i.e. ‘cryosaunas’). The neuromuscular effect of WBC may also be significant. It has been proposed that cooling-induced alterations in muscle spindle activity can contribute to neuromuscular function changes (Westerlund *et al.,* 2009). A decrease in flight time during drop jump tests was observed following single WBC treatments, yet this decrease was reversed following repetitive WBC application, indicating neuromuscular adaptation and reduced co-activation of the agonist and antagonist muscles (Westerlund *et al.,* 2009). Indeed, neuromuscular function can directly influence the force and rate of muscle contraction via the activation of motor units (Kenney *et al.,* 2015). The possible impact of cryotherapy on muscular performance shall be discussed in section 2.3.5.

2.2.3. Anti-Inflammatory activity

The anti-inflammatory effect of cryotherapy treatment may be significant for promoting muscle recovery following strenuous exercise due to its relation with EIMD mechanisms. As covered previously (section 2.1), EIMD is characterised by a pronounced inflammatory response involving leukocyte infiltration into damaged tissue and the upregulation of pro-inflammatory cytokines (e.g. TNFα and IL-1β). This may result in further degradation of muscle tissue, amplifying the damage (Ferreira-Junior *et al.,* 2014). It has been theorised that extreme cold can attenuate the activity of soluble intercellular adhesion molecule 1 (sICAM-1), an important molecule involved in the recruitment and adhesion of leukocytes to muscle tissue, a key event in the initial inflammatory response post-exercise (Ferreira-Junior *et al.,* 2014). However, recent findings have rebutted the theory that WBC treatments induce substantial decreases in sICAM-1 levels in the blood (Bieuzen *et al.,* 2019). Despite the general lack of evidence for mitigated sICAM-1 activity following singular WBC treatments, cryotherapy can modify the pro-inflammatory versus anti-inflammatory cytokine balance in favour of the latter, as demonstrated by Banfi *et al.* (2009).

An anti-inflammatory effect of WBC has been identified by Pournot *et al.* (2011), who observed a significant increase in the anti-inflammatory cytokine IL-1 receptor antagonist and a decrease in pro-inflammatory cytokine IL-1β when WBC was applied following a simulated trail run. These effects were subsequently corroborated when WBC was applied following a 30 minute step exercise (Ziemann *et al.,* 2013). However, such studies utilised multiple treatments of WBC post-exercise which are

likely to induce a more potent anti-inflammatory effect than a single treatment. Krueger *et al.* (2018) recently reported no significant impact of a single WBC treatment on inflammatory markers after a high intensity running bout. Thus, the precise effectiveness of WBC in curtailing the inflammatory response post-exercise remains contentious.

The effect of cryotherapy on the cytokine interleukin-6 (IL-6), an important mediator of the body's inflammatory response (McGeough *et al.,* 2012) remains unclear. Since IL-6 can have both pro and anti-inflammatory effects (Hody *et al.,* 2019), the meaningfulness of any impact of WBC on IL-6 response is open to interpretation. Ziemann *et al.* (2012) and Lubkowska *et al.* (2011) discovered increases in IL-6 following several WBC treatments over a several day period. Conversely, Mila-Kierzenkowska *et al.* (2013) discovered a decrease in IL-6 levels following a single WBC treatment and Pournot *et al.* (2011) found no effect. Therefore, it remains unclear what the precise effect is of WBC on IL-6 response post-exercise as well as performance implications.

Another reported anti-inflammatory effect of WBC is the stabilisation (i.e. reduced activity) of lysosomal enzymes, the activation of which can contribute to the muscle breakdown process after strenuous or muscle damaging exercise (Wozniak *et al.,* 2007). Lysosomal activity is associated with autophagy, the degradation of cellular structures, which can contribute to muscular atrophy (Sandri, 2013). The research addressing the role of lysosomal enzymes and/or activity in relation to EIMD is scant, however it is conceivable that mitigating this via WBC could benefit muscle function post-exercise due to reduced muscle breakdown.

2.2.4. Reactive Oxygen Species and Oxidative Status

Damaging exercise is characterised by the pronounced production of reactive oxygen species which can contribute to the destabilisation of myofibrillar structures, potentially reducing force generation capacity (White & Wells, 2013). The body's oxidative status (i.e. balance between pro-oxidant and anti-oxidant activity) could be another physiological characteristic by which cryotherapy influences muscle damage alleviation and recovery. The body's anti-oxidant defence is reflected by its capacity to counter the effects of reactive oxygen species. An anti-oxidant enzyme that has been commonly examined to gauge the pro. vs anti-oxidant balance is superoxide

dismutase (SOD). It has been demonstrated that applying WBC as a stand-alone treatment results in increased SOD activity (Miller, 2012). Yet, applying WBC treatments over several days in conjunction with a training load has been reported to cause a decrease in SOD activity (Mila-KierzenKowska *et al.,* 2009; Wozniak *et al.,* 2007). It is possible that any decrease in anti-oxidant levels and/or activity post-WBC is a consequence of adaptive homeostatic mechanisms, suggestive of an improved overall anti-oxidant capacity following multiple treatments (Mila-KierzenKowska *et al.,* 2009; Zembron-Lacny *et al.,* 2020). Although cryotherapy may have pronounced anti-oxidative effects post-strenuous exercise, it is not clear whether this is a substantial contributing factor to accelerating muscle recovery, or indeed enhancing athletic performance.

2.2.5. Autonomic Nervous System and Cardiovascular Effects

WBC can significantly affect the autonomic nervous system (ANS) with concomitant effects on recovery. The potential underlying mechanism is thought to be the adrenergic activation of receptors in the vascular wall. Hausswirth *et al.* (2013) revealed a pronounced increase in plasma noradrenaline post-WBC, reflective of activation of sympathetic α-adrenergic fibres and the ensuing cutaneous vasoconstriction. There was also an increase in heart rate variability indices, indicative of enhanced parasympathetic control of heart rate, which potentially promotes recovery and has a cardioprotective effect (Hausswirth *et al.,* 2013). It is claimed that the enhanced parasympathetic recovery following WBC post-exercise is associated with baroreflex activation, CIVD and an increased central blood volume (Bleakley *et al.,* 2014). Furthermore, WBC has been demonstrated to immediately increase and decrease blood pressure and heart rate respectively (Lubkowska & Szygula, 2010). More recent findings have supported these observations in reporting reduced heart rate and ventilation during an interval running session post-WBC compared to passive recovery (Piras *et al.,* 2019).

2.3. Issues associated with whole body cryotherapy for sports recovery

Despite the known physiological and biochemical research, the true effectiveness of WBC treatment remains contentious and further studies are required to determine the efficacy of WBC as a treatment method for sport and exercise recovery and justify its use. Some discussion issues associated with this are exercise-induced muscle damage, comparisons with other forms of cryotherapy, psychological and neuromuscular effects and sleep.

2.3.1. Exercise-Induced Muscle Damage

EIMD is an extensively researched topic in the field of exercise physiology with several comprehensive reviews available (Clarkson & Hubal, 2002; Hody *et al.,* 2019; Owens *et al.,* 2018; Peake *et al*., 2017). As mentioned previously (section 2.1), significant muscle damage can occur as a result of mechanical strain induced by extensive eccentric contractions. Excessive stretching of the sarcomeres beyond the myofilament overlap causes them to become “popped” and the time course of EIMD based on peak sarcomere disruption typically varies from 1-3 days, but may occur for longer depending on the severity of exercise (Peake *et al.,* 2017). Several forms of exercise have the potential to cause substantial EIMD such as interval sprints (Wiewelhove *et al.,* 2015) and eccentric contraction dominant protocols such as downhill running (Dolci *et al.,* 2015; Malm *et al*., 2004), drop jumps (Hohenauer *et al*., 2017; Miyama & Osaka, 2004) and isolated eccentric leg extensions (Costello *et al*., 2012; Vieira *et al*., 2015). Whilst these exercise protocols have high potential to substantially induce muscle damage, the precise mechanisms may vary depending on the nature of the exercise. For instance, the extent of mechanical strain imposed upon muscles and consequently potential time course of damage can be determined by a number of factors. These include number of stressed muscle groups, extent and torque of eccentric contractions, speed of contractions, range of motion and joint angle velocity, amongst others (Paulsen *et al.,* 2012; Peake *et al.,* 2017). Although inconclusive, it is conceivable that the specific muscle damage mechanisms following a standardised exercise bout (e.g. extent of leukocyte infiltration, myofibrillar disruption etc.) could influence how effective any intervention is for recovery.

It would be prudent for any study examining EIMD and recovery in further depth to consider the occurrence of the repeated bout effect (RBE). This is a natural phenomenon whereby the muscle is more resistant to successive bouts of muscle damaging exercise due to adaptations, manifested by alleviated soreness, swelling, reductions in muscle torque and quicker recovery to baseline (Hody *et al.,* 2019). The RBE has been reported to last up to 6 months (Nosaka *et al.,* 2001).

An important consideration is whether WBC is a potent means of alleviating EIMD. It is considered that the most reliable marker of muscle damage is force production (Warren *et al.,* 1999). As mentioned previously (section 2.2.2), cryotherapy can have significant positive effects on torque output post-exercise (Fonda & Sarabon, 2013; Hausswirth *et al.,* 2011), which would have beneficial consequences for athletic performance. On the contrary, other WBC studies reveal a lack of positive outcomes on muscle strength post-exercise (Russell *et al.,* 2017; Wilson *et al.,* 2018). The differences in exercise protocols may explain the discrepant findings.

Whilst the analgesic effect of cryotherapy is relevant from a physiotherapeutic perspective, it can substantially contribute to the alleviation of DOMS following arduous exercise, a well-established feature of EIMD. Despite the reported effects in the literature (Fonda & Sarabon, 2013), some of which are summarised in Figure 2.2.1, a review has suggested there is insufficient evidence to determine if WBC effectively treats DOMS (Costello *et al.,* 2015).

It is possible that WBC can benefit exercise recovery independent of alleviating muscle soreness. One common marker of EIMD is creatine kinase (CK), an enzyme that leaks into the circulation as a consequence of damaged muscle tissue (Clarkson & Hubal, 2002). Should WBC be associated with a reduced CK leakage, this may indicate an enhanced muscle fibre repair and reduced breakdown and/or permeability of the muscle cell membrane. The findings regarding the effect of WBC on plasma CK levels are inconclusive. Some studies have revealed an impact of WBC on plasma CK (Banfi *et al.,* 2009; Ziemann *et al.,* 2012), whereas others have revealed no effect (Hausswirth *et al.,* 2011). However, the validity of using CK as a measure of muscle damage may be questionable. CK levels in the plasma are determined not only by the extent of muscle fibre damage, but also by the rate of clearance from the blood (Brancaccio *et al.,* 2007), making it more difficult to accurately interpret CK levels during the recovery period. Additionally, the nature of the exercise bout may affect the time course of recovery, thus impacting CK

clearance from the blood (Clarkson & Hubal, 2002). Another factor to consider is the specific isoenzyme of CK. There are three distinct tissue specific CK isoenzymes, dependent on where they are derived from: MM from skeletal muscle, MB from the heart and BB from the brain (Baird *et al.,* 2012). There are also two specific forms of CK expressed in the mitochondria (Mt-CK) - ubiquitous Mt-CK and sarcomeric Mt-CK (Baird *et al.,* 2012). It follows that in the context of muscle damage induced by exercise, it would be beneficial to solely assess the MM isoenzyme. Since muscle damage studies generally do not specify the isoform analysed within the blood samples, the origin of plasma CK may be ambiguous and there is a possibility of inter-individual variability in CK levels due to the different isoforms. Findings in plasma CK levels post-muscle damaging exercise and recovery protocols should therefore be interpreted with caution.

The potential impact of WBC in the treatment of EIMD remains a contentious issue within the literature and the sporting community in general. This has been addressed further in one of the studies presented in this body of work (chapter 4).

2.3.2. Comparison with other forms of Cryotherapy

Considering the commonly adopted practice of protocols for CWI and ice baths (some effects described in section 2.1), a question that remains unanswered is whether WBC treatment is superior for the purpose of sports recovery. To address this question, the overall effectiveness needs to be considered, as well as economic viability and accessibility. The majority of sports individuals would not have immediate access to a WBC unit and some treatments may entail a considerable journey beforehand. Additionally, CWI is easier to prepare and access as well as being considerably cheaper. The comparison of physiological and performance effects between the two methods is clearly of importance. CWI can similarly induce increases in Interleukin-6 (IL-6), indicating a potential anti-inflammatory effect (Roberts *et al.,* 2014). Moreover, the reactive hyperaemic effect (introduced in section 2.2.1) following vasoconstriction has also been demonstrated following CWI applications (Gregson *et al.,* 2011). There are however, conflicting findings regarding whether CWI alleviates the degree of EIMD, with some studies (Ascensao *et al.,* 2011; Bailey *et al.,* 2007; Rossato *et al.,* 2011) revealing an attenuation of muscle torque losses and DOMS following a standardised exercise bout, with others (Crystal *et al.,* 2013; Goodall & Howatson, 2008) not reporting such benefits.

One factor to consider when comparing cryotherapy effects is the specific exercise protocol. Studies such as Bailey *et al.* (2007) have adopted shuttle runs, whereas others (e.g. Fonda & Sarabon, 2013) have applied multiple eccentric contractions specifically designed to induce muscle damage. The mechanisms of EIMD may differ between various exercise bouts, therefore the induced stress might be mechanical or metabolic in nature, which can influence the effectiveness of cryotherapy treatment (Leeder *et al.,* 2012). It is proposed that resistance exercises with predominantly eccentric contractions, for example eccentric leg curls emphasising the 'negative' phase of the contraction, will produce mostly mechanical stress. Conversely, steady state or high intensity exercises are more associated with metabolic stress (White & Wells, 2013). As mentioned prior (section 2.3.1), muscle damage can be caused by a variety of exercise types. Thus, it would be beneficial to account for such factors when determining the effectiveness of both WBC and CWI treatments.

One notable difference between WBC and CWI is that the latter imposes a hydrostatic pressure, which can magnify the fluid replacement effect, contributing to reducing oedema and enhancing waste removal (White & Wells, 2013). This was later supported by Hayter *et al.* (2016), who discovered that CWI at 14°C benefited anaerobic cycling performance for up to 24 hours post-strength training exercises for the lower body musculature, compared to cold air therapy at the same temperature. It could therefore be argued that the effect of hydrostatic pressure in CWI compensates for the fact that WBC has a superior thermal gradient. Moreover, the thermal conductivity of the cold air of WBC is inferior to that of ice/water applications (Bleakley *et al.,* 2014).

Several studies have directly compared effects of WBC with CWI. Costello *et al.* (2012) examined differences in skin and muscle temperature responses between the two methods. It was discovered that muscle and core temperature both decreased to similar extents following the two cryotherapy modes, however WBC elicited a more pronounced knee skin temperature decrease. Based on this, it could initially be argued that WBC is more effective for obtaining an analgesic effect. However, CWI was later discovered to induce more prominent decreases in leg blood flow and thigh skin temperature post-cycling bout compared to WBC (Mawhinney *et al.,* 2017), which contrast the findings of Costello *et al.* (2012). The earlier study only utilised a water immersion duration of four minutes whereas the latter used 10 minutes, which is arguably more akin to real sports practice. It is also worth noting that the earlier

study did not assess thermoregulatory responses of cryotherapy post-exercise, which may be a factor contributing to the discrepant findings.

It was also concluded that CWI caused superior physiological effects compared to partial body cryotherapy (PBC) at −135°C following a drop jump protocol (Hohenauer *et al.,* 2017). However, other aspects within the study would imply favourable use of PBC. Firstly, muscle torque appeared to recover faster for PBC at 48 hours post-exercise compared to CWI. Secondly, vertical jump performance was superior for PBC within an hour post-exercise. Thirdly, PBC curtailed muscle swelling more than CWI, which contradicts the theory that CWI can mitigate swelling to a larger extent due to the hydrostatic effect. Thus, there may be a discrepancy between physiological effects and performance outcomes.

Similarly, Abaidia *et al.* (2017) claimed that CWI resulted in superior recovery than WBC (following eccentric hamstring curls) based on kinetics in countermovement jump performance, soreness and perceived recovery. However, it appears that isometric force production was somewhat superior for WBC than CWI at 24 hours post-treatment, which may have attained significance had the study benefited with a larger sample size. Jump performance was also similar between the two conditions up to 48 hours post-treatment. Abaidia *et al.* (2017) also adopted a cross-over design, meaning that participants underwent the exercise protocol twice within a short timespan (two week wash out period), thus were subject to experience the repeated bout effect, which could partially invalidate the results. This is a phenomenon whereby muscle damage is less severe following a second exercise bout due to adaptations occurring following the first bout (Dolci *et al.,* 2015; Miyama & Nosaka, 2004).

A marathon race has also been used to compare recovery characteristics between the two cold modalities where WBC resulted in more detrimental recovery with regards to muscle torque production and plasma CK (Wilson *et al.,* 2018). However, WBC appeared to benefit muscle soreness and stress perception. The same research group also reported contradictory findings when comparing WBC with CWI post-resistance exercise in a subsequent study (Wilson *et al.,* 2019), with some variables (e.g. muscle strength) indicating more benefits from WBC, whilst there were non-beneficial or negative effects with other variables (e.g. countermovement jump height). It should be noted that the unconventional cryotherapy protocol (two short

bouts of −85°C) used in their studies makes it difficult to draw tenable comparisons and conclusions in the context of the wider literature.

Despite the studies by Abaidia *et al*. (2017), Hohenauer *et al.* (2017) and Wilson *et al*. (2017) suggesting that CWI is superior to WBC/PBC, the overall consensus is equivocal since some variables indicate that CWI is more beneficial than WBC, whereas other variables indicate the opposite. Therefore, the conclusions from these studies should be interpreted with caution.

More recently, a Chinese research group discovered WBC to be more beneficial than CWI in muscle performance, soreness and inflammatory markers over multiple days following a 90 minute treadmill run (Qu *et al.,* 2020). It is clear that there is no established consensus as to which of the two cold treatments is superior for supporting muscle function, performance measures and thereby post-exercise recovery. The discrepant findings are likely due to several reasons, notably differences in exercise protocols, cold treatment protocols (e.g. frequencies, durations, temperatures), specific measures utilised as well as variability in participant characteristics. Any beneficial impact of WBC must also be balanced with the economic implications (see chapter 8).

2.3.3. Psychological Effects

Since an athlete's mental state can have an impact on their performance, the potential psychological influence of WBC should be considered. Benefits to mental health have been reported in several patients with spinal pain, peripheral joint disorders and multiple sclerosis based on psychological well-being index questionnaires following repetitive WBC applications (Pawik *et al.,* 2019; Szczepańska-Gieracha *et al.,* 2014).

Psychological effects of WBC following exercise have also been reported, such as better sense of well-being (Hausswirth *et al.,* 2011), however the literature directly investigating the psychological impact of WBC in sports recovery contexts is limited. Whilst cryotherapy can induce a variety of physiological responses to promote recovery from arduous exercise training, a study by Broatch *et al.* (2014) proposed that such benefits are related to the mental perception of a beneficial effect. The research discovered that immersing participants in a thermoneutral water bath at 35°C (applied with skin cleanser to lead the participants to believe that the treatment

would benefit recovery), following high intensity cycling sprints recovered muscle torques comparably to 10°C immersions. This occurred despite the intramuscular temperature difference observed between the two water bath conditions. There were also benefits reported for participants' pain reduction and readiness for exercise following the placebo condition. Such findings present a case against the efficacy of cryotherapy for enhancing muscle recovery, but whether the placebo effect can also be inferred for WBC remains unclear. Future studies which assess psychological outcomes following WBC treatments applied as a post-exercise recovery modality are warranted.

2.3.4. Sleep

It would be beneficial to consider the implications of WBC on sleep. Several athletes and coaches are concerned with ensuring sufficient sleep between hard training sessions and/or competition to promote swift recovery. The importance of sleep is undisputed (Walsh *et al.,* 2020), yet in the context of sports recovery, several factors such as protein synthesis, hormone regulation, carbohydrate metabolism and alertness necessitate sleep further (Halson, 2015). Schaal *et al.* (2014) found that elite female swimmers' sleep duration and efficiency was significantly enhanced during a fortnight of intense training in conjunction with daily WBC treatment compared to a control period. It was suggested that the swimmers' perceived fatigue was lower during the WBC treatment period (Schaal *et al.,* 2014). Despite these promising findings, there were no significant benefits to swimming performance and speed.

Further studies have shown that WBC interventions can enhance sleep quality in a variety of physically active populations, thus promoting recovery and reducing fatigue (Bouzigon *et al.,* 2014; Douzi *et al.,* 2018; Douzi *et al*., 2019). However, the direct link between cryotherapy-induced benefits in sleep and subsequent performance remains dubious. There is clear scope for further developments in this area since the impact on sports performance could be considerable.

2.3.5. Neuromuscular Effects

It is theorised that cold application can enhance muscle power due to increasing motor unit recruitment and/or impulse discharge rate (Lubkowkska, 2012). However, the research supporting this is equivocal. Studies have looked at muscle performance parameters post-cryotherapy, with Fonda and Sarabon (2013) demonstrating beneficial effects on muscle power through vertical jump tests. However, Ascensao *et al.* (2011) and Vieira *et al.* (2015) reported no benefits to power tests following cold treatments post-muscle damaging exercise. Possible explanations for the discrepancy are the nature and severity of the exercise bouts and resultant damage. For instance the study by Vieira *et al.* (2015) utilised purely unilateral contractions of knee extensors, whereas Fonda and Sarabon (2013) applied a combination of resistance and power based exercises on both legs. The specific context and purpose of cryotherapy application could also impact the findings. Vieira *et al.* (2015) aimed to assess whether WBC could effectively be used to promote same day muscle recovery. This would be particularly relevant for sports participants training or competing twice on the same day. Neuromuscular changes could also be mediated by cold-induced alterations in muscle spindle activity (Westerlund *et al.,* 2009), as mentioned in section 2.2.2

2.4. The effects of WBC on performance

WBC may induce a wide array of physiological parameter changes post-exercise, however it is not clear if these can be directly transferred to functional measures and sports performance. For example, the precise implication of a reduction in tissue temperature for athletic performance remains unanswered. Whilst it has been reported that tissue temperature changes is a determining factor affecting subsequent physiological responses and recovery (White & Wells, 2013), the relationship between such alterations and subsequent performance output remains unclear.

Despite the diverse literature examining WBC effects in athletic contexts, there is a lack of studies identifying definitive associations between physiological effects post-WBC and subsequent muscle function/sports performance parameters. Ziemann *et al.* (2012) attempted to demonstrate a potential link between physiology and

performance with their study on professional tennis players. Repetitive WBC treatment over 5 days resulted in numerous significant changes during a subsequent tennis drill. Blood cytokine profiles were altered to reflect more anti-inflammatory activity; oxygen uptake and heart rates were reduced to suggest lower physiological cost and more efficient aerobic function; and stroke effectiveness was markedly improved compared to a control group (Ziemann *et al.,* 2012). Each of these alterations demonstrates a clear physiological benefit and enhanced recovery post-cryotherapy in a performance context. Although this finding may have potential widespread implications, no correlations were undertaken between the physiological and performance parameters. It would thereby be difficult to conclude that the improved stroke effectiveness was a direct cause of the physiological changes induced by the repetitive WBC treatment, or due to a higher sense of wellbeing or freshness. It could also be argued that specific benefits to tennis cannot be extrapolated to other sports with differing demands.

The benefits of cryotherapy for acute recovery and endurance performance is not clear. Peiffer *et al.* (2010) demonstrated superior acute recovery during a cycling bout following CWI, although this was examined in hot conditions. The literature addressing this question for thermoneutral environments is scant. However, it has been demonstrated that WBC treatment can induce benefits to acute muscle recovery and running performance, whereby several variables such as muscle oxygenation and VO_2 during running bouts were examined (Kruger *et al.,* 2015). These parameters were increased and decreased respectively during a repeated exercise test, indicating enhanced running economy and therefore performance (Kruger *et al.,* 2015). Whilst WBC can induce reductions in core temperature (Costello *et al.,* 2012), it remains dubious whether this substantially contributes to performance gains. A cooler body core can enhance exercise tolerance, enabling skeletal muscles to contract at the desired force output and delay fatigue accordingly (Marino, 2002). It seems plausible that this can benefit sports performance, particularly for same day recovery. It has been reported that WBC induces rectal temperature reductions that can be sustained for a period after cold exposure removal (Westerlund *et al.,* 2003). This was supported by Costello *et al.* (2012) who revealed rectal temperature reductions of around 0.3°C 60 minutes after WBC. Despite attaining statistical significance ($p<0.05$), the clinical or athletic significance of this reduction is debatable. Yet Kruger *et al.* (2015) discovered that core temperatures were approximately 0.2°C lower in participants who had undergone

WBC post-exercise compared to participants undergoing passive recovery, prior to performing a repeated treadmill running test 15 minutes post-WBC. With a large effect size of 0.86, it might be reasonable to assume that the difference in core temperature was a contributing factor to the clear difference in performance observed between the two groups during the repeated treadmill test. However, this is contentious since at least one of several other assessed variables could also have been a factor (e.g. muscle oxygenation, reduction in cardiovascular strain, sensation of recovery) and no analysis or correlations were undertaken to examine the relationship between core temperature and exercise performance. Therefore, causal effect was not established.

Further research would be beneficial to directly associate core temperature reductions with performance gains. The study by Kruger *et al.* (2015) demonstrates that WBC can enhance same day recovery through the assessment of physiological parameters and endurance performance. Sports participants could apply this to support their performance when they are training and/or competing repeatedly in quick succession.

2.5. The conflict between optimising recovery benefits and training adaptations

Whilst cold applications might be used to restore function and accelerate recovery post-exercise, therefore potentially facilitating subsequent training loads, it is possible that the acute effects of cold exposure can interfere with regenerative processes necessary to support chronic adaptations, particularly if applied repetitively. The research addressing the potential issue of whether long term and repetitive cryotherapy treatment can paradoxically hinder chronic adaptations to training is neither clear nor abundant. It has previously been shown that following a training protocol over several weeks, increases in blood flow and arterial vessel diameters (measured using ultrasonogrophy) were less evident for repetitively cooled limbs via CWI than limbs that were not cooled post exercise (Yamane *et al.,* 2006), indicative of attenuated adaptations. It was suggested that the stimulus required for myofibre regeneration and consequently muscle hypertrophy can be prevented by repetitive and chronic cryotherapy, thus jeopardising long term training adaptations (Yamane *et al.,* 2006).

An additional study demonstrated that repetitive CWI application over several weeks resulted in attenuated anabolic signalling mechanisms following a resistance training programme, with clear attenuations in muscle strength and mass development. (Roberts *et al.,* 2015). The proposed underlying mechanism is that the reduced muscle blood flow post-cryotherapy reduces amino acid delivery, thereby reducing activation of signalling pathways (Roberts *et al.,* 2015). One notable example is the mammalian target of rapamycin (mTOR), a significant pathway in regulating muscle protein synthesis and subsequently hypertrophy post-exercise (Haff & Triplett, 2016; Roberts *et al.,* 2015).

Such CWI studies would indicate that repetitive cold exposure can blunt beneficial adaptations to training. As mentioned prior, muscle damaging exercise bouts causes a pronounced inflammatory response characterised by infiltration of leukocytes into muscle tissue and consequent upregulation of pro-inflammatory cytokines, which act in concert to degrade muscle tissue further, thus amplifying the initial damage (Peake *et al.,* 2017). It is worth considering the potential extent to which repetitive WBC treatments might curtail the extensive inflammatory response that commonly occurs following muscle damaging exercises, which are integral to muscular repair and regeneration (Peake *et al.,* 2017). A WBC-induced mitigation of EIMD/inflammation could be counter-productive for adaptive changes in the long term.

The possible flow of processes leading to reduced muscle hypertrophy and strength development is summarised in Figure 2.5.1. On the contrary, work has supported the notion that cryotherapy may enhance chronic adaptations to training through the discovery of an increase in muscle gene expression of PGC1α, a key regulator in the process of mitochondrial biogenesis (Ihsan *et al*., 2014). This could have positive implications for long term adaptations to oxidative capacity.

Four studies to date have studied responses to a training programme in conjunction with repetitive WBC treatments. Broatch *et al.* (2019) discovered that multiple WBC treatments did not impact endurance adaptations to a 4 week cycling programme involving weekly high intensity interval sessions. When compared to a control group, Jaworska *et al.* (2018, 2021) noted growth factor benefits in volleyball players and judo fighters following a two week period incorporating repeated WBC and sport specific exercises. A recent study revealed that repetitive WBC can support muscle hypertrophy gains via a reduction in myostatin levels, an established negative mediator of muscle adaptations (Jaworska *et al.,* 2020). These studies would

therefore indicate no negative consequences of repetitive WBC application on adaptive responses, in contrary to some of the CWI studies. Further studies assessing the influence of multiple WBC applications on long term adaptations to training would be of interest to sports individuals motivated to enhance performance. The possible influence of the frequency of WBC treatments will be examined in further detail in section 2.6.4.

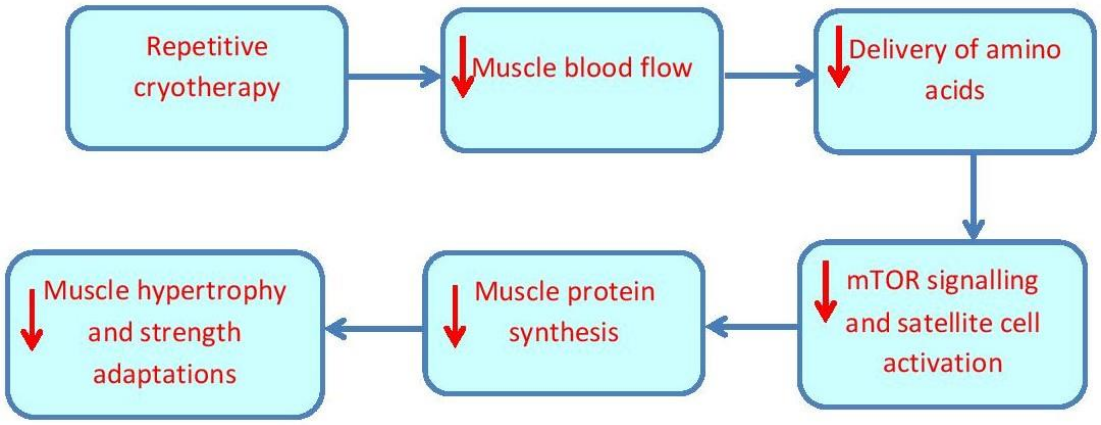


Figure 2.5.1: Flow chart demonstrating potential mechanism by which repetitive cryotherapy treatments can hinder adaptations to training. mTOR - mammalian target of rapamycin, an important signalling pathway implicated in muscle hypertrophy (Roberts *et al.,* 2015)

The question that sports scientists, athletes and coaches need to ask is whether the benefit of WBC in enhancing recovery and subsequent quality of sessions outweighs the possible detriment to adaptation. This 'recovery vs adaptation paradox' could apply for endurance, power and/or resistance based training. Having a better understanding of this question could have implications for cryotherapy applications in relation to the periodisation of a training schedule. It is worth noting that the effectiveness or hindrance of cryotherapy treatments for long term adaptations can depend on the specific stage of an athlete's training and/or competition. For instance, there might be variations in emphasis between recovery from training or performance during competition. Although speculative, cryotherapy may be particularly appropriate at a stage when athletes are most fatigued or stressed from training (such as an 'overreaching' phase). Moreover, repetitive cryotherapy treatments might benefit athletes most when attempting to recover between hard competitions in quick succession during a competitive phase, or during a tapering phase, since achieving

training adaptations is unlikely to be the athlete's top priority. No research thus far has suggested that training adaptations acquired throughout a preparation phase can be lost by undertaking repetitive cryotherapy treatments during the midst of a competitive phase.

Another factor that could affect how training adaptations are influenced by repetitive cryotherapy is the nature of the exercise training. Based on the findings of Yamane *et al.* (2006), Roberts *et al.* (2015) and Ihsan *et al.* (2014), it would be reasonable to speculate that cryotherapy is more likely to hinder long term gains where exercises are predominantly geared towards resistance training and the desired outcome is improvements in muscle strength and/or hypertrophy. The literature also generally indicates lack of negative consequences of repetitive cold exposure on endurance performance (Broatch *et al.,* 2019; Halson *et al.,* 2014). Factors such as sample sizes, the length of training periods and training statuses of participants could partially explain the discrepant findings.

The issue of frequent WBC treatments potentially hindering adaptations to training is therefore one that warrants further evaluation. This shall thereby be explored and addressed further in the final study within this thesis (chapter 6).

2.6. Establishing optimal procedures for whole body cryotherapy

The optimum protocol for WBC treatment in terms of duration, temperature, frequency and timing is debatable, a notion shared by others (Bleakley & Hopkins, 2010; Costello *et al.,* 2012). Whilst several researchers have utilised standardised protocols (see sections 2.6.1 to 2.6.4) that have been adopted by earlier studies, it remains unclear precisely which protocol would lead to the optimal physiological and biochemical benefits, muscle recovery and sports performance. This could be an importance consideration due to the expensiveness and rarity of the treatment.

2.6.1. Timing

One intriguing factor that could influence the effectiveness of cryotherapy treatment, which has not been addressed in the literature to date, is whether the timing of

treatment affects the response. More specifically, at what stage of recovery post-exercise WBC can still confer beneficial effects. The majority of studies to examine the positive effect of WBC on muscle function and recovery applied treatment within 15 minutes post-exercise, with various findings, as covered previously. Other studies have applied the treatment significantly later. Kruger *et al.* (2015) and Fonda and Sarabon (2013) both reported some benefits to recovery using WBC 45 minutes and one hour post-exercise respectively. However, Costello *et al.* (2012) revealed that applying WBC 24 hours after a muscle damaging exercise bout had no beneficial effect on muscle torque or DOMS for up to three days following a single treatment. It is conceivable that the initial 24 hour period after strenuous exercise represents a window by which cryotherapy can intervene positively to influence the progression of muscle damage and loss of force production capacity. The acute inflammatory response, such as infiltration of leukocytes, is prominent immediately after damaging exercise and is proposed to correspond to the initial or primary muscle damage, contributing to the ensuing secondary muscle damage and pronounced losses in muscle function over subsequent days (Ferreira-Junior *et al*., 2014). It has been reported that a local muscular pro-inflammatory response precedes a systemic blood anti-inflammatory response (Peake *et al.,* 2005) and myofibrillar disruption is most prominent 24 hours post-damaging exercise (Paulsen *et al.,* 2012). This may explain why treatments being applied after 24 hours are too late to provide recovery benefits. Thus, there may be a specific time point during the recovery window whereby cryotherapy can be applied to produce meaningful or optimal recovery. In order to maximise the benefits of WBC, it may be necessary to apply the treatment when the inflammatory process is at its most potent in order to curtail inflammation to promote restoration of muscle function. Logistically, it may not be practical for individual and team sport athletes to undergo WBC treatment immediately after hard training or competition, with factors such as cool down, nutrition, transport and treatment availability meaning a wait of a few hours may be necessary before treatment. The comparative impact of cryotherapy throughout different stages of this critical recovery period warrants further investigation, hence this issue has been addressed in one of the studies in this body of research (chapter 5).

2.6.2. Temperature

The majority of WBC studies utilise temperatures in the range of -110°C to -140°C (Table 2.7.1) and there are discrepant findings in recovery responses, with benefits being reported on both ends of the spectrum. For instance, benefits following single -110°C treatments have been reported (Ferreira-Junior *et al.,* 2014; Ferreira-Junior *et al.,* 2015; Kruger *et al.,* 2015), whilst Fonda and Sarabon (2013) reported some recovery benefits (e.g. jump power) using treatments in the range of -140°C to -185°C.

'Warmer' WBC treatment temperatures have also been applied in the literature, notably -85°C (Wilson *et al.,* 2018) in their marathon race study (section 2.3.5) and even -40°C (Douzi *et al.,* 2018), revealing significant benefits in their study on sleep quality. Due to these unconventional temperatures, it is difficult to define the 'extreme cold' temperature range that is characteristic of WBC treatments.

Due to the superior thermal gradient and potential for heat exchange, it would be reasonable to hypothesise that more extreme cold temperatures would more profoundly affect physiological responses, such as reductions in skin and muscle temperatures. It is acknowledged that there have been no studies assessing the effect of manipulating WBC treatment temperatures alone, making it difficult to draw definitive conclusions on the influence of this factor for sports recovery. Additionally, it is probable that there are discrepancies in the reported temperatures of WBC chambers and actual temperatures whilst the subjects undergo their treatments, as indicated in the review by Bouzigon *et al*. (2016). Whilst small fluctuations in chamber temperature during treatments are inevitable, this would also make it difficult to conclude the precise impact of treatment temperature manipulations on physiology and performance outcomes.

To summarise, due to the short duration of treatments, it is unlikely that temperature plays a significant role in influencing the efficacy of WBC for post-exercise recovery. For future studies and treatments, it would be sensible to stay within the -110°C to -140°C range.

2.6.3. Duration

Whilst the majority of WBC treatments are typically three minutes long (Costello *et al.,* 2012; Hausswirth *et al.,* 2013; Kruger *et al.,* 2015), it has been suggested that WBC durations exceeding 2.5 minutes may not be necessary due to the added thermal discomfort (Fonda *et al.,* 2014). Other researchers have attempted to identify the optimum treatment duration. Selfe *et al.* (2014) compared between three different WBC durations (-135°C) as recovery interventions following rugby matches. They concluded that a two minute exposure may be ideal taking into account thermal comfort and physiological measures such as muscle tissue oxygenation and skin temperatures. One limitation noted in the study was the occurrence of 'peripheral vascular shutdown' immediately following cryotherapy, making it difficult to withdraw reliable blood samples and observe changes in haematological markers. Furthermore, the rationale for selecting a two minute exposure for future treatments as opposed to three minutes is questionable on the premise that skin temperatures were significantly lower immediately post-cryotherapy for three minutes duration than two minutes, whilst other variables (including thermal comfort) were not significantly different between these two durations. Taking into account the literature, whether such subtle differences in treatment duration have a significant impact on physiological and performance parameters is contentious.

2.6.4. Frequency

Regarding optimum cryotherapy practices, it is worth considering the potential significance of the frequency of treatments. Whilst single treatments of WBC may not reflect the standard practice of athletes, it is likely to be more economical and feasible than multiple treatments, particularly for non-professional athletes who do not have extra funding. Studies have applied multiple treatments post-exercise (Pournot *et al.,* 2011) to address whether repetitive WBC treatment in quick succession over several days is significantly more impactful than a single treatment, arguably displaying superior results. It has been hypothesised (Pournot *et al.,* 2011) that multiple WBC treatments can have an accumulative effect on blunting acute phase inflammation following exercise bouts. More specifically, the testosterone to cortisol ratio may be enhanced (Grasso *et al.,* 2014), which can have further implications for subsequent sports performance, particularly during 'overreaching' phases where athletes are training maximally and significantly increasing their

overload, necessitating greater recovery. An enhanced testosterone to cortisol ratio may represent a more anabolic state, impacting protein synthesis as well as muscle glycogen replenishment (Urhausen *et al.,* 1995) which might be more conducive for muscle growth and development. Furthermore, overtraining can be prevented in the long term due to modifications in the body's hormonal asset (Grasso *et al.,* 2014), which could enable athletes to establish greater control over their training schedules and recovery processes. The question of whether the dose of cryotherapy can have an overall impact on exercise recovery and training adaptations has not been clarified, although it has been shown that a higher treatment frequency can augment acute responses such as anti-inflammation (Lubkowska *et al.,* 2011), anti-oxidant capacity (Lubkowska *et al.,* 2012) and post-occlusive hyperaemia (Renata *et al.,* 2011). Further research in this area would be beneficial.

2.6.5. Inter-individual factors

It is worth noting that the exact optimum procedure for WBC can largely depend on inter-individual factors determining the response. Studies have demonstrated that adipose tissue content is inversely related to the magnitude of muscle tissue cooling owing to its insulating effect (Myrer *et al.,* 2001). Similarly it has been revealed that a higher skinfold thickness (a common measure of body fat levels) significantly increases the time required to decrease intramuscular temperature by a set amount (Otte *et al.,* 2002). This is due to the insulation properties of adipose tissue owing to the reduced magnitude of conductive heat transfer from the body core to the external surrounding (Bleakley & Davison, 2010), therefore potentially reducing the overall effect of cold exposure. Coaches and clinical practitioners should consequently be mindful of the body compositions of athletes and patients before applying cryotherapy treatments to support recovery, performance and/or injury repair. Moreover, the age of WBC users would be an additional consideration since functional decline associated with ageing could impact the treatment effectiveness. A reduced responsiveness of the ageing blood vessels (Knight & Nigam, 2008) may be significant in the context of cryotherapy, because of the potential impact on CIVD, blood redistribution and heat transfer. These two factors should not be considered independent of each other when assessing response to exercise and recovery methods, as adipose tissue content naturally increases with age due to a reduction in basal metabolic rate (Kenney *et al.,* 2015).

The research on sex differences in cryotherapy responses is generally limited. However, two studies have clearly demonstrated that females experienced more pronounced skin temperature reductions than males following single WBC treatments (Cuttell *et al.,* 2017; Hammond *et al.,* 2014) which could partially be explained by their higher body fat contents. Combining their finding with the discovery that higher adiposities also attenuate muscle temperature reductions, the overall implication of body adiposity for post-exercise recovery is equivocal. It is possible that individuals with higher body fat would retain internal core and tissue temperatures to a larger extent following cryotherapy treatments owing to a reduced CIVD response compared to leaner individuals. This could jeopardise their ability to recover from exercise accordingly, although no studies to date have examined sex differences in WBC responses for recovery post-exercise. It would be useful to clarify which factors can influence the treatment effect of WBC and would enable users to be classified as 'high or low responders'. The inter-individual variability of response to WBC treatment could be closely related to the reported inter-individual variability in EIMD response post-eccentric exercise (Paulsen *et al.,* 2012; Yoon & Kim, 2020). Additionally, sex differences could also be a factor in responses to muscle damaging exercises, since higher estrogen contents in women could confer a protective effect (Hohenauer *et al.,* 2020). Therefore, there may be individual variation in the response to WBC dependant on the inter-individual factors associated with severity of muscular damage and the possible mechanisms associated with such damage.

In either case, the influence of such inter-individual factors on responses to cryotherapy post-exercise is an issue that has not been addressed fully in the literature. It is anticipated that the study findings presented in chapter 4 provides further insight into the potential role of age and body fat content on responses to cryotherapy.

2.7. Conclusions and Implications

WBC is an emerging method of sports recovery with numerous physiological effects, yet the verdict remains contentious as to whether it is an overall effective recovery method. Such studies by Pournot *et al.* (2011), Hausswirth *et al.* (2012) and Ferreira-Junior *et al.* (2015) advocate the use of WBC for enhancing recovery following

training. The effectiveness of WBC in enhancing recovery depends partially on the specific characteristics of the exercise bout, such as intensity, duration and whether the exercise stress is predominantly mechanical or metabolic in nature.

Further research is required to make more definitive conclusions regarding the sports performance implications of such bodily responses. Such conclusions can be supported by further clarification of the effect of certain factors. Firstly, it would be reasonable to assume that the timing of treatments can impact the effectiveness of cryotherapy because of the specific mechanisms of recovery during the post-exercise period. In addition, the inter-individual variability of responses to WBC should not be ignored. Further, it is of interest to a variety of individuals to elucidate the precise effectiveness of WBC treatment for promoting recovery from strenuous exercise, training and/or sports competition, particularly when cold water baths are readily available. Finally, the question of whether long term repetitive WBC treatments can hinder adaptations to training is an intriguing one that warrants further investigation.

Table 2.7.1 provides a comprehensive summary of whole body cryotherapy studies assessing different variables post-exercise indicating either beneficial or non-beneficial effects.

Table 2.7.1: Summary of studies assessing variable response following WBC treatment post-exercise, with indications of positive effects or no effects. * PBC – Partial Body Cryotherapy where the head is not directly exposed to cold. ** - Westerlund et al. (2009) discovered no benefit to muscle power after a single WBC treatment, but neuromuscular adaptations were noted after 3 months. *** Studies by Abaidia et al. (2017), Hohenauer et al. (2017), Mawhinney et al. (2017) and Qu et al. (2020) only compared WBC effects to CWI, with no control group. # Wilson et al. (2019) used different methods of assessing muscle strength, with contradictory outcomes regarding WBC.

Study	Sample characteristics	Exercise protocol/s	WBC protocol	Variables measured	Major findings
Hausswirth et al., (2011)	9 runners, average age 31.8	48 minute simulated trail run on treadmill (alternating gradients)	3 mins at -110°C, 3 times	Isometric muscle torque, muscle pain, CK, mental wellbeing	+ve effect on muscle torque, pain and wellbeing
Fonda & Sarabon (2013)	11 males, active, average age 26.9	Drop jumps and leg curls.	3 mins at -140°C - -185°C for 7 consecutive days	Power and torque tests, pain, CK	+ve effect on muscle torque, jump power and pain
Ferreira-Junior et al., (2014)	12 males, active , average age 23.9	Concentric and eccentric knee extensions – 6 sets of 10 repetitions for each	3 mins at -110°C (PBC*)	Concentric and eccentric muscle torque	+ve effect on muscle torque
Ferreira-Junior et al., (2015)	26 males, active, average age 20.2	Drop jumps – 5 sets of 20 repetitions	3 mins at −110 °C (PBC*)	Isometric muscle torque, muscle soreness, muscle thickness	+ve effect on muscle torque, muscle thickness
Costello et al., (2012)	18 Ps – male and Female	Eccentric knee extensions – 20 sets of 5 repetitions	3 mins at -110°C	Isometric muscle torque, muscle soreness, cycling sprint test	No +ve effect
Westerlund et al., (2009)	10 males and 4 females, average age 33	Drop jumps	2 mins at -110°C, 3 times a week for 3 months	Muscle power, maximal arm contractions, stretch reflex, EMG of calf muscles	No +ve effect after single treatment, but neuromuscular adaptation noted after 3 months **
Vieira et al., (2015)	12 males, average age 23.9, resistance trained	Concentric and eccentric knee extensions – 6 sets of 10 repetitions	3 mins at -110°C	Vertical jump power, skin temp	No +ve effect
Banfi et al., (2009)	10 male rugby players, average age 26	Regular rugby training	2 mins at -110°C after 30 secs at -60°C, for 5 consecutive days	CK, cytokines, sICAM*	+ve effect on CK, cytokines (e.g. anti-inflammation)

Ziemann et al., (2012)	12 male tennis players	5 days moderate intensity training – strength, endurance, agility.	3 mins at -120°C, twice a day for 5 consecutive days	VO2, IL-6, CK, tennis drill	+ve effects on inflammation, CK, VO_2, heart rate
Wozniak et al., (2007)	21 kayakers, male and female, average age 24 years.	10 days of training – endurance, strength and water	3 mins -120°C - -140°C, 3 times for 10 consecutive days	CK, variety of lysosomal enzymes	+ve effect on CK activity (i.e. reduced activity) after 6 days training
Pournot et al., (2011)	11 male runners, average age 31.8	48 minute simulated trail run on treadmill (alternating gradients)	3 mins at -110°C for 4 consecutive days	Cytokines, leukocytes	+ve effect on cytokines (i.e. anti-inflammation)
Selfe et al., (2014)	14 male rugby players, average age 24.	Rugby fixture	1, 2 or 3 mins at -135°C, after 30 secs at -60°C	Cytokines, tissue oxygenation, core temp, skin temp	No effect on cytokines, +ve and –ve effect on tissue oxygenation.
Kruger et al., (2015)	11 male athletes, average age 26	5 x 5 minutes of high intensity running	3 mins at -110°C	Tissue oxygenation, VO2, core temp, skin temp, heart rate	+ve effect on tissue oxygenation, VO2.
Wozniak et al., (2007)	20 elite male kayakers, 10 untrained men.	Kayak training, including strength and endurance training	3 mins at -120 - -140°C after 20 sec at -60°C over 3 sessions for 10 days (trained). 3 mins at -120°C (untrained)	Pro and anti-oxidants	+ve effect on anti-oxidants (e.g. superoxide dismutase)
Ziemann et al., (2013)	18 males, average age 22	30 minute step ups	3 mins at -110°C after 30 sec at -60°C, twice a day for 5 consecutive days	IL-10, IL1β, CK, muscle soreness, cholesterol.	+ve effect on inflammation, CK, DOMS, cholesterol (LDL/HDL ratio)
Schaal et al., (2014)	10 elite female swimmers, average age 20	2 week intense swimming training	3 mins at -110°C every day during training period.	Blood lactate, salivary α-amylase, cortisol, sleep	+ve effect on salivary α-amylase, blood lactate, sleep duration and sleep efficiency.
Grasso et al., (2014)	25 male rugby players, average age 25.6	Rugby training	3 mins at -140°C after 30 sec at -60°C, twice a day for 7 consecutive days	Salivary cortisol, testosterone	+ve effect on cortisol and testosterone
Russell et al., (2017)	14 Premier League academy football players	15 x 30m sprints	2 mins at -135°C after 30 sec at -60°C	Muscle power output, soreness, plasma CK, testosterone	+ve effect on testosterone. No effect on other variables.

Abaidia et al., (2017)	10 males, active, average age 23.4	Eccentric knee flexions – 5 sets of 15 repetitions.	3 mins at -110°C	Isometric muscle torque, soreness, CK, countermovement jumps.	No +ve effect. ***
Hohenauer et al., (2017)	19 males, average age 25.9	Drop jumps – 5 sets of 20 repetitions	2 mins at -135°C after 30 sec at -60°C (PBC*)	Maximal leg extension, soreness, muscle swelling, skin temp, muscle oxygenation.	Small +ve effect on vertical jump, muscle swelling, torque ***
Mawhinney et al., (2017)	10 males, average age 22.3	Steady state cycling until core temp 38°C reached (average 45 mins)	2 mins at -110°C	Skin and muscle temps, leg blood flow.	Reduction in muscle temperature, heart rate ***
Wilson et al., (2018)	10 male runners, average age 37.7-41.3.	Marathon race	3 mins at −85°C and 4 mins at −85°C, separated by 15 mins	Isometric muscle torque, soreness, drop jump, CK, cytokines,	-ve effect on muscle torque, drop jump.
Krueger et al., (2018)	11 male athletes, average age 25.9.	5 x 5 minutes of high intensity running	3 mins at -110°C	Cytokines, testosterone, cortisol	No +ve effect
Douzi et al., (2018)	12 active males, average age 28.5	55 minute running session of different intensities	3 mins at -40°C	Sleep quality, pain, skin temp	+ve effect on sleep quality
Douzi et al., (2019)	9 male football players, average age 24.8	90 minute training session – football, interval running and plyometrics	90 sec – 3 mins at −180 °C (PBC*)	Sleep efficiency and duration, skin temp	+ve effect on sleep quality
Wilson et al., (2019)	24 males, resistance trained, average age 24.8	Lower body resistance exercises – 120 repetitions in total.	3 mins at −85°C and 4 mins at −85°C, separated by 15 mins	Isometric muscle torque, soreness, CMJ, isometric squat	+ve and –ve effects on torque. #
Piras et al., (2019)	9 rugby players, average age 23.7.	Resistance exercises	3 mins at −160 °C (PBC*)	Blood lactate, heart rate, VO_2, ventilation	+ve effects on HR, VO_2, ventilation
Qu et al., (2020)	12 male runners, average age 20.0	90 minute treadmill run of alternating gradients and drop jumps (20 sets of 40 reps)	3 mins at -110°C – -140°C, 4 times	Soreness, CK, vertical jump	+ve effects on soreness and CK
Hohenauer et al., (2020)	28 active females, average age 22.5	Drop jumps – 5 sets of 20 repetitions	2 mins at -135°C after 30 sec at -60°C (PBC*)	Isometric muscle torque, soreness, swelling, skin temp, vertical jump	+ve effect on soreness. No effect on muscle torque, jump, swelling.

CHAPTER 3 –

GENERAL METHODS AND PILOT STUDIES

3.1. Introduction

The methods and measurement techniques used repeatedly throughout the thesis are discussed in this chapter, including a description of the whole body cryotherapy chamber. The reliability of methods are also discussed where appropriate.

Pre-Test Information and Considerations

Ethical approval was obtained from the Research Ethics Committee of the Graduate School at the University of Northampton. Risk assessments (Appendix 1) were completed in the principal locations for testing: University of Northampton Park campus, University of Northampton Waterside campus and Chris Moody Centre, Moulton College. Prior to testing, subjects were asked to read participant information sheets and were given the opportunity to ask questions. They received verbal explanations of all study procedures in question and reminded of their right to withdraw at any stage. A written consent form was signed (Appendix 4). A medical questionnaire was completed which detailed all medications and illnesses (Appendix 3). Responses were assessed against contra-indications to exercise participation and whole body cryotherapy treatment (Appendix 2). Conditions included hypertension, defined as a blood pressure exceeding 140/90 according to the British Medical Journal. Resting systolic and diastolic blood pressures were measured in triplicate using a blood pressure monitor (Omron 773, West Sussex) after participants were seated for 10 minutes. The mean values of the three readings were calculated. Participants were asked to refrain from alcohol and strenuous physical activity for 24 and 48 hours prior to testing respectively.

3.2. Measures

Anthropometry

Height and body mass were measured on the participants' first visit to the laboratory. Scales were used for the measurement of body mass (Seca, Germany) and body height (Seca, Birmingham, UK). Body fat content was assessed by skinfold calipers

(Harpenden/British Indicators, West Sussex, UK) according to guidelines set by the International Society for the Advancement of Kinanthropometry (ISAK). Four skinfold sites were used: Biceps, triceps subscapular and iliac crest. The total skinfold thickness from the four sites were summed and converted to an estimated body fat percentage based on a conversion table (Baty British Indicators). The skinfold technique has been demonstrated to be a reliable method for assessing body fat, with comparable or superior results to standard bioelectrical impedance devices (Gualdi-Russo *et al.,* 1997; Loennekke *et al.,* 2013) and has been used in previous cryotherapy studies concerned with body composition (Hammond *et al.,* 2014)

Isometric Muscle Torque

Unilateral isometric muscle torque was assessed by an isokinetic dynamometer (Biodex Medical Systems 3, New York) calibrated during initialisation prior to each study. The dynamometer was fitted with a lever arm attachment with a shin pad locked in at an angle of 90º leg extension. Data generated by the dynamometer were transferred to a Biopac data acquisition system (Biopac, CA, USA) via an analogue input.

Participants sat on the dynamometer chair in an upright position with approximately 90° of flexion at the hips. The chair was adjusted so that the pivot of the lever arm was located directly adjacent to the lateral epicondyle of the right leg. The right leg was strapped to the lever arm attachment with the bottom of the protective shin pad located just superior to the medial malleolus of the ankle (Figure 3.2.1). The following parameters were noted for all future muscle torque assessments: Back rest, chair length, chair height and lever arm length. The shoulders, trunk and right thigh were strapped tight to avoid excessive upper body motion (Ferreira-Junior *et al.,* 2015). Participants were asked to place their arms across their chests without gripping hold of the shoulder straps.

Figure 3.2.1: Standardised positioning of participants on the Biodex Dynamometer.

For familiarisation, participants performed two submaximal contractions at 60% and 80% subjective effort respectively, followed by two maximal contractions, with verbal encouragement given throughout. Each contraction lasted 5 seconds in duration and there was a 30 second recovery period between each. During each contraction they exerted force against the pad with their right leg. They were required to minimise movement in other regions of their body and were advised to exhale during each contraction. For maximal torque assessment, unilateral isometric maximal voluntary contractions (MVC) of the right leg quadriceps occurred as follows. Participants performed two submaximal warm up contractions of slight increments in force with 30 second recoveries between each. This was followed by a two minute rest period. They then performed 4 maximal contractions (with two minute recovery between each), each contraction lasting 5 seconds in duration, with verbal encouragement given throughout (Dolci *et al.,* 2015). The peak torque (Nm) was determined as the maximum of the 4 contractions. Previous studies have revealed high levels of reliability and validity in the Biodex dynamometer (de Araujo Ribeiro Alvares *et al.,* 2015; Lund, 2005) with a test-retest reliability correlation coefficient of 0.91 reported by Ferreira-Junior *et al.* (2015). Furthermore, a pilot study conducted in the laboratory (see section 3.4) revealed a day to day variance of 5.3% within individuals.

Muscle Soreness

With the exception of pilot study 3 (section 3.6), subjective muscle soreness was reported via one visual analogue scale (VAS) assessment (Gould *et al.,* 2001, Appendix 5). This involved participants squatting with their back against a wall and knees flexed to a 90º angle. This position was held for three seconds and participants marked on the scale with a cross how much pain they felt in their upper legs from "no pain" to "pain as bad as it could possibly be". The distances marked on the scales were measured in millimetres then converted to a percentage. Despite VAS being subjective, it is a common assessment for muscle soreness post-eccentric exercise (Fortes *et al.,* 2013; Glasgow *et al.,* 2014), with high reliability demonstrated in indicating pain severity (Yin Lau *et al.,* 2013).

Creatine Kinase

Creatine kinase (CK) is considered to be the most common blood marker of muscle damage (Clarkson & Hubal, 2002). Assessments of CK were conducted by fingerstick sampling using lancets. CK was analysed using a Reflotron Plus analyser (Oberoi Consulting, Derby). To ensure appropriate calibration prior to testing, a clean and check strip was inserted into the machine and the readings were assessed against reference ranges on the label. 30µl of whole blood was obtained from the fingertip of the participant's middle finger, transferred to the test strip and inserted into the machine to reveal the reading. Resting reference values for whole blood samples are 24.4 – 1400 IU/L. A reliability coefficient of 3.1% has previously been reported for CK using the Reflotron system (Horder *et al.,* 1991). The second pilot study (section 3.5) revealed an 11.7% within subject variance. Plasma CK levels are reported to be affected by clearance rate from the blood (Brancaccio *et al.,* 2007) and CK exists in various tissue-specific isoenzymes (Baird *et al.,* 2012.

Interleukin-6

Whole blood samples were obtained by venepuncture into 6ml Ethylenediaminetetraacetic acid (EDTA) vacutainer tubes (BD Diagnostics, Oxford). Following separation via centrifuge at 2000g for 10 minutes, neat blood plasma was transferred into 1.5ml eppendorf tubes and frozen at -80ºC for subsequent analysis.

Concentrations of IL-6 in thawed samples were quantified by Bio-Plex Multiplex Immunoassays (Bio-Rad, UK), a technique that utilises dual fluorescent polystyrene beads. The IL-6 detection limit was 0.49pg/ml. A high reliability of the multiplex immunoassay system has been demonstrated previously, particularly compared to other immune-analytical techniques such as enzyme-linked immunosorbent assays and with mean co-efficient of variance (CV) values as low as 2.8% (Fu *et al.,* 2010; Tighe *et al.,* 2013).

Haemoglobin

Haemoglobin (Hb) was quantified by a 'hemocue' haemoglobin monitor (Hemocue, Sweden) following the fingerstick technique to obtain whole blood, according to manufacturer's instructions. This measure can gauge haemolysis and the oxygen carrying capacity of blood, providing an indicator of efficiency of oxygen delivery. The hemocue monitor has commonly been used as a quick and reliable assessment for haemoglobin in clinical trials and exercise studies (Jaggernath *et al.,* 2016), and is considered to have high accuracy compared to laboratory reference values (Sanchis-Gomar *et al.,* 2012).

Skin Temperatures

Non-contact thermal imaging cameras (FLIR systems, Sweden) were used for the assessment of skin temperatures. Human skin emits an infrared signature based on its temperature, which can be detected and quantified by the camera. The camera can detect temperatures up to a range of 650°C and within sensitivities of <0.07°C, on several regions of the body. An accuracy of ± 0.2°C has been demonstrated with an emissivity setting of 0.95 (Tanda, 2015). Images were captured whilst the camera was held 3.0 metres from the participant and set at an emissivity factor of 0.98. Built in software (FLIR Tools+) was used to record and store thermal images. The thermographs produced by this software were analysed based on pre-established regions of interest (via a box measurement tool) on the chest (c), anterior thighs (t), posterior upper arms (a) and calves (ca). The mean skin temperature was calculated by a commonly used equation:

$Ts = 0.3 (c + a) + 0.2 (t + ca)$ (Ramanthan, 1964).

Thermal imaging cameras have routinely been used for the assessment of skin temperatures pre and post-WBC treatment (Costello *et al.,* 2013; Selfe *et al.,* 2014) and is regarded as a highly accurate and reliable tool for non-invasive measures of human skin temperatures (Costello *et al.,* 2012).

Core Temperatures

Tympanic temperatures were assessed by a thermometer (Braun Thermoscan 7) inserted into the right ear canal. A disposable pre-warmed tip was applied to the end of the thermometer prior to each measurement in order to reduce the cooling effect of the thermometer probe in the ear. The mean reading from three recordings was used for data collection purposes. The thermometer measures the infrared heat generated by the eardrum and surrounding tissue. Due to the eardrum sharing blood supply with the temperature control centre in the hypothalamus of the brain, the ear is purported to accurately reflect the core body temperature with studies demonstrating a comparable level of accuracy to rectal measurements (Childs *et al.,* 1999; Purssell *et al.,* 2009). In addition, tympanic temperature monitoring is less invasive and discomforting for participants.

Oxygen Consumptions and Maximum Aerobic Capacity Assessments

For maximal oxygen consumption (VO_2 max) assessments, an online breath by breath analyser (Cortex Metalyser, Germany, Figure 3.2.2) measured concentrations of oxygen and carbon dioxide. The Cortex was calibrated prior to each assessment. This was to ensure that the expected gas concentrations for oxygen and carbon dioxide indicated on the machine matched the concentrations of gases derived from gas cylinders (Cranlea, Birmingham, UK), which were routinely checked and maintained, ensuring no leakages. To enable calibration of the Cortex analyser, the ambient temperature, humidity, gas flow volume and ambient pressure were inputted into the analyser prior to each assessment. The Cortex analyser was calibrated accordingly by connecting it with oxygen and carbon dioxide gases derived from the cylinders. The flow volume was calibrated by a syringe (Figure 3.2.2), ensuring that the speed of pumping was synchronised with a rhythm bar on the screen. The cortex analyser was stored in stable conditions to ensure minimal pressure fluctuations. A built in software (Metasoft) enabled graphs and readings of the participant's oxygen

and carbon dioxide consumption to be displayed on screen in real time. The Cortex Metalyser has been used in several previous exercise studies (Dolci *et al.,* 2015; Fortes *et al.,* 2013) and is considered a reliable and valid tool for assessing exercise intensities, with high intra-class reliability coefficients of 0.97 and 0.96 for oxygen and carbon dioxide reported respectively (Meyer *et al.,* 2001).

Figure 3.2.2: Cortex Metalyser with accompanying syringe to calibrate flow volume.

Maximal aerobic capacity was assessed via an incremental treadmill (Cosmos, Germany) protocol comparable to the Bruce protocol (Bruce, 1963; Figure 3.2.3). Speed was initially progressed every two minutes by 2km/h from a starting speed of 6km/h until 16km/h was reached. Thereafter, gradient was increased by 2% each two minute stage (Table 3.2.1). An appropriately sized and fitted face mask was worn by the participant ensuring that the mask was connected to the Cortex analyser via a gas tube and gas turbine. Prior to running, resting oxygen and carbon dioxide consumptions were measured to ensure the system was working appropriately. Rating of perceived exertion and heart rates were recorded at the end of each stage. Oxygen and carbon dioxide were analysed by the gas analyser. The assessment stopped once the participant experienced volitional exhaustion. As well as reaching volitional exhaustion, two conditions confirmed that participants had reached their maximum aerobic capacity: 1) a respiratory quotient of 1.10 or higher; 2) a VO_2 plateau of 150ml/min or less between consecutive stages. VO_2 max is widely regarded as the gold standard marker of aerobic capacity (Jones & Carter, 2000). Several cryotherapy studies have effectively utilised VO_2 max protocols to assess cardiorespiratory fitness as well as subsequent exercise intensities (Hausswirth *et al.,* 2011; Ziemann *et al.,* 2012). Similar incremental treadmill protocols have also demonstrated high levels of reliability and validity for VO_2 max assessments (Weltman *et al.,* 1990).

Table 3.2.1: Protocol for VO_2 max assessments. Speed was increased in small increments. Gradient was increased if participants successfully completed the 6th stage.

Stage	Speed (km/h)	Gradient (%)
1 (0-2 mins)	6	1
2 (2-4 mins)	8	1
3 (4-6 mins)	10	1
4 (6-8 mins)	12	1
5 (8-10 mins)	14	1
6 (10-12 mins)	16	1
7 (12-14 mins)	16	3
8 (14-16 mins)	16	5
9 (16-18 mins)	16	7

Figure 3.2.3: Participant performing maximal aerobic capacity test to assess VO_2 max. Facemask is linked to Cortex gas analyser.

Doppler Ultrasound

Resting femoral artery blood flow was assessed via ultrasound using a 12L-RS linear transducer probe (frequency range 5-13MHz) attached to an ultrasound machine (Vivid I, GE Medical Systems, Israel, Figure 3.2.4) in the pulse wave Doppler mode. Images were taken at an insonation angle of 60° at the superficial femoral artery of the right thigh approximately 3cm inferior from the bifurcation with the deep femoral artery. Longitudinal B-mode images of the lumen-arterial wall interface were optimised. Each measurement in the pulse wave was recorded for 30 seconds. Images were stored and later analysed. Arterial diameter was measured perpendicularly between the lumen-intima interfaces of the superior and inferior walls of the artery. Arterial diameters and velocities were determined as the mean of three 30 second pulse wave measures. Mean blood velocities of each trace were measured from the velocity time integrals on the machine.

Overall leg blood flow (LBF) was calculated using the following equation:

LBF (ml/min) = 60 x Mean blood velocity (cm/s) x π x artery radius^2 (Blanco, 2015)

Participants were rested in a supine position for at least 5 minutes before images were taken and were asked to avoid caffeine, alcohol and strenuous exercise for at least 4, 24 and 48 hours respectively prior to assessments. All measurements were taken by the same operator to avoid inter-rater experimental bias.

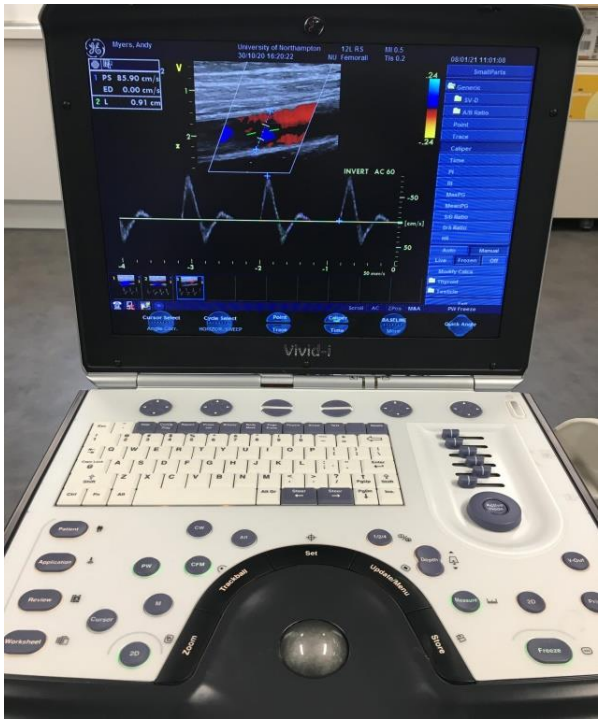


Figure 3.2.4: Vivid I ultrasound machine to assess femoral artery diameters and blood velocities. Image displayed in pulse wave mode.

Rating of Perceived Exertion

Participants reported subjective intensities by a rate of perceived exertion (RPE) 15 point scale (Borg, 1989, Appendix 8) at regular intervals. Overall RPE was reported, with a combination of central and local exertion ratings requested from participants. Whilst oxygen consumptions are generally regarded as a more accurate means of assessing exercise intensities, RPE indicates subjective exertion, thereby being useful to assess participants' comfort and potential risk of withdrawing from running protocols. RPE has also been shown to be a reliable and valid assessment for exercise intensities, with reasonably strong correlations with other intensity markers, including percentage of VO_2 max, heart rate and blood lactate concentration (Chen *et al.,* 2002; Scherr *et al.,* 2013).

Heart Rate

Heart rates (HR) were measured via belts strapped to participants (Polar Electro, Finland) with the conductor located on the bottom of the sternum. The conductor uses electrocardiography to detect the electrical signal in the heart. A microprocessor inside the conductor on the chest belt records the HR based on this signal and synchronises this with the display on an accompanying monitor watch, which records the HR at regular intervals. Despite being potentially influenced by external factors such as stress, hydration status and environmental variables, Polar HR monitors are routinely used for accurate and valid measures of HR and can be used to predict energy expenditure during exercise (Crouter *et al.,* 2004). Combined with oxygen consumptions and rating of perceived exertion, HR provide a convenient and thorough assessment of exercise intensities and can correlate closely with percentage of VO_2 max at submaximal intensities (Achten & Jeukendrup, 2003).

Thermal Comfort

Subjective thermal comfort was reported via a visual analogue scale (VAS) assessment (Appendix 6). Participants were asked to report a number between -3 (uncomfortably cold) and +3 (uncomfortably hot).

Wellbeing

A visual analogue scale (Appendix 7) was used to assess participants' subjective mental wellbeing and overall sense of satisfaction. Participants marked a cross on the scale depending on their overall wellbeing from "I do not feel comfortable, healthy and satisfied" to "I feel extremely comfortable, healthy and satisfied". The distances marked on the scales were measured in millimetres then converted to a percentage.

3.3. Interventions

Whole Body Cryotherapy Treatment

Cryotherapy treatments were undertaken in a two-stage cryogenic chamber (JUKA, Poland) routinely calibrated and serviced annually. The source of cold was liquid cryogenic gas originating from external pressure vessels. Risk assessments were carried out prior to treatments. The cryogenic chamber was switched on for a minimum period of 30 minutes prior to participant entry for the temperature to descend to the required level. This included the activation of ventilation and dehumidifier. Thereafter, the internal temperatures remained constant for the duration of activation. Participants were screened for contraindications prior to testing (Appendix 2). Before entering the chamber, participants wore a head band, face mask, two pairs of gloves, two pairs of socks, elbow and knee bands, as well as two pairs of clogs. Verbal instructions were then given, including an explanation of the procedure and safety guidelines. Participants entered the cryotherapy chamber with initial exposure to a vestibule chamber at -60°C undertaken for 30 seconds, followed by the main chamber at -120°C for 150 seconds. This temperature was selected as a compromise between the range of -110°C (Ferreira-Junior *et al.,* 2014; Hausswirth *et al.,* 2011) to -140°C (Fonda & Sarabon, 2013; Grasso *et al.,* 2014) previously used in WBC studies. Participants were advised to keep mobile during the whole 3 minute period. On completion, the exit door for the main chamber opened, allowing participants to exit (Figure 3.3.1). Thereafter, participants were advised to stay mobile before changing in usual clothing within 5 minutes. The cryogenic chamber was then deactivated and dried off, including activation of a safety stop switch to disconnect the machine from the power supply.

Figure 3.3.1: Participant exiting the cryotherapy chamber after completion of 3 minute treatment

Muscle Damaging Exercise Protocols - Downhill running

Participants were asked to stand on a motorised treadmill (HPCosmos, Germany) facing the reverse direction. The treadmill was set at a 15% gradient decline (10% for pilot studies 1 and 3, sections 3.4 and 3.6). A warm up walk at a speed of 5 km/h was performed for two minutes, enabling participants to adjust to the treadmill. The treadmill speed was then gradually increased and adjusted so that the participants' average HR during the 30 minute run was roughly equivalent to their target HR extrapolated from 60% of their VO_2 max. For the pilot studies, subjects' maximal heart rates were estimated by the equation HR_{max} = 202 - (0.55 x age) (Whyte *et al.,* 2008). The total duration of downhill running was 30 minutes (20 minutes for pilot study 1, section 3.4). The 30 minutes commenced as soon as the participant started running - i.e. treadmill belt was moving too fast for the participant to walk. Invariably, this was after the participant started walking, but earlier than attaining the target heart rate. Rating of perceived exertions (Borg, 1989) and heart rate were monitored at 2, 4, 5, 10, 15, 20, 25 and 30 minutes. The participant was closely supervised with verbal encouragement given throughout. Downhill running has commonly been

employed in previous studies inducing and assessing muscle damage (Fortes *et al.,* 2013; Malm *et al.,* 2004).

Figure 3.3.2: Participant performing 30 minute downhill run at 15% decline gradient.

Muscle Damaging Exercise Protocols - Resistance Exercises

For the alternative muscle damaging protocol in pilot study 3 (section 3.6), participants performed a series of resistance exercises targeted on the lower limbs, predominantly using free weights (Eleiko, Sweden). The protocol included movements chosen to induce metabolic and mechanical damage on the muscle fibres. Exercises were as follows, with 60 seconds of recovery between sets.

- Dumbell lunges – 3 sets of 8 repetitions on each leg at 70% of one repetition maximum (1RM)

- Barbell squats – 3 sets of 8 at 70% 1RM

- Knee extension – 3 x 10 at 70% 1RM

- Nordic leg curls - 2 x 8

- Depth jumps – 2 x 10 from a step height of 30cm.

One Repetition Maximum Protocol

In order for the correct load to be used for the resistance exercise protocol in pilot study 3, participants' one repetition maximum was determined for the barbell squat, dumbbell lunges and knee extension. Following a warm up consisting of between 5-10 submaximal contractions for each exercise (depending on how accustomed participants were to exercises and technique), the weight was gradually increased until participants attained the maximum weight they could complete the exercise with correct form for one repetition. The weight increments were 2.5kg to 10kg for the squat, 2.5kg per dumbbell for the lunge and 10kg for the leg extension. Participants were given at least a two minute recovery period between each maximal attempt. For both the barbell squat and dumbbell lunge, participants were required to descend until their quadriceps were parallel to the ground and to fully extend at the knees on the upward phase. The correct technique was ensured during the warm up and demonstrated where necessary. Verbal encouragement and close supervision was provided throughout.

3.4. Pilot Study 1 – Reliability of muscle torque and downhill running

Aims

Maximal muscle torque was anticipated to be the primary marker of muscle damage post-exercise throughout the studies undertaken in this body of work. The primary aim of this pilot study was to compare and evaluate the effectiveness of two selected downhill running protocols to induce muscle damage. Such downhill runs occurred at one of two sites – University of Northampton Park campus (PC) and the Chris Moody Centre (CMC) of Moulton College which differed according to the decline gradient: 10% and 3% respectively. For the purpose of the main studies, conducting the muscle damaging exercise protocol at the CMC would be preferable owing to close proximity to the cryotherapy chamber. This would also ensure tighter control over the timing that WBC treatments can be applied post-exercise, which as discussed earlier is a potentially important consideration (section 2.6.1).

The secondary aim of this pilot study was to assess the reliability of maximal muscle torque measures within individuals (i.e. day to day variability) and between sessions using the isokinetic dynamometer. This enabled determination of how many

familiarisation sessions would be necessary for the individuals to become accustomed to the muscle torque protocol. It was also important for familiarity and practical competence to be developed to enable accurate use of the dynamometer. It was hypothesised that the downhill run at Park campus would cause exercise-induced muscle damage and that such damage would not be prominent following the treadmill run at the Chris Moody Centre.

Methods

Participants:

Twelve male participants aged 18 or over (mean ± SD age 35.3 ± 15.3 years, height 1.78 ± 0.1m, body mass 77.8 ± 7.2 kg, body mass index 24.7 ± 1.6 kg/m^2) were recruited for this pilot study. All were fit, healthy and free from cardiovascular or neural conditions. These were determined through a medical questionnaire (Appendix 3) and pre-testing measures of blood pressure, height and body mass. A full verbal explanation of the study was provided to all participants who provided written consent.

Protocol:

Participants were asked to perform two trials in a randomised crossover design, with a total of six sessions. This involved four laboratory sessions for the first trial (PC/CMC) and twice for the second trial (PC/CMC). The first visit entailed familiarisation and a baseline assessment of muscle torque of the right quadriceps. The second visit occurred 24 hours after the first visit and involved a further baseline assessment of muscle torque. The third visit occurred 24 hours after the second visit and involved a third baseline assessment of muscle torque followed by a muscle damaging exercise bout. The fourth visit was an assessment of muscle torque and soreness 24 hours post-muscle damaging exercise. The final two sessions occurred at least 5 days later and assessed muscle torque and soreness pre and post-running at the other trial site.

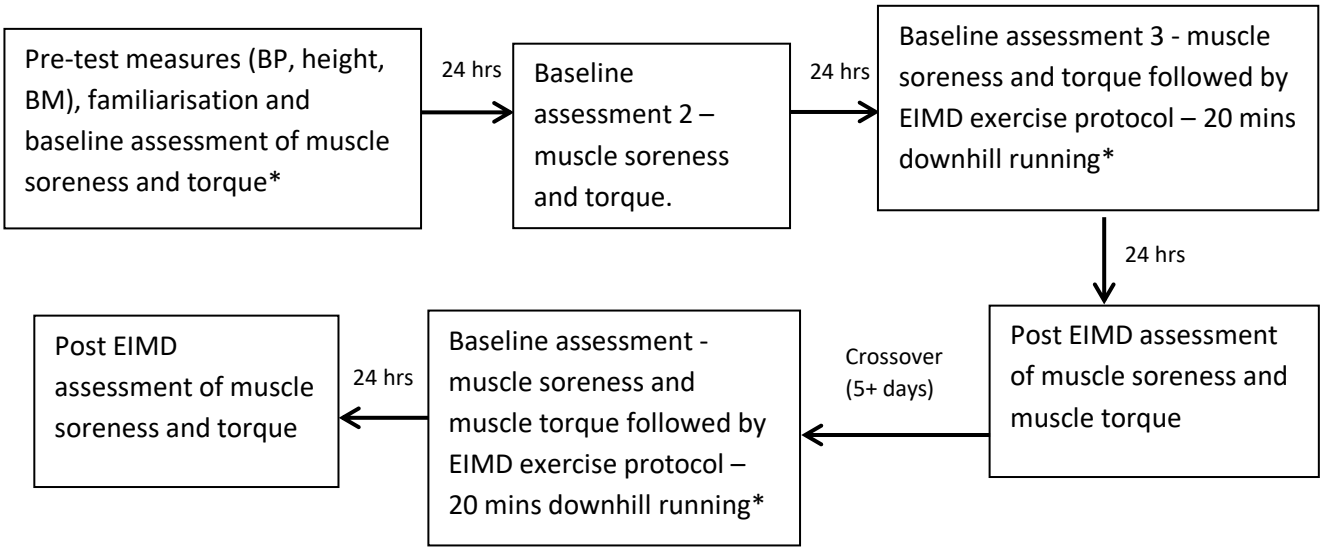
For the first visit prior to muscle torque assessment and following 10 minutes of rest in a seated position, participants' resting systolic and diastolic blood pressure was measured as described in section 3.1. Participants discovered to be hypertensive (blood pressure exceeding 140/90 mm/Hg) were excluded from the study. Height and body mass were also measured. Participants were first familiarised with the Biodex dynamometer, including the provision of clear verbal instructions, as described in section 3.2. Subjective muscle soreness was reported via a visual analogue scale as described in section 3.2 (squatting against a wall). Participants were then repositioned and re-strapped into the dynamometer for assessment of unilateral isometric maximal voluntary contractions (MVC) of the right leg quadriceps using the procedure as described in section 3.2. The peak torque was determined as the maximum of the four contractions.

For the second and third laboratory visits occurring 24 and 48 hours after the first visit respectively, participants underwent further assessments of their muscle soreness and baseline MVC torques with the same procedures. The comparison of the first three muscle torque assessments was intended to determine the variability of torque outcomes and the overall coefficient of variance. During the third session, the MVC protocol was followed 20 minutes later by the exercise-induced muscle damage protocol. This involved 20 minute downhill running at an intensity of 70% of maximal heart rate, as described in section 3.2. The downhill run occurred on either the treadmill at PC (10% gradient decline) or the CMC (3% decline). Participants were then allowed to leave the laboratory.

For the fourth session, participants returned to the laboratory 24 hours post-downhill run to undertake a muscle soreness and maximal muscle torque assessment. The change in muscle torque and muscle soreness from the measures obtained prior to downhill running was used to assess the severity of muscle damage.

At least 5 days was given to participants before conducting their crossover trial to eliminate effects of fatigue. For session 5, participants completed the same protocol as described for the third session, except the treadmill run took place in the different site. Participants returned to the laboratory 24 hours later to complete their final session, involving a muscle soreness and muscle torque assessment, with the same protocol as that of session 4.

Schematic of sessions for pilot study 1:



* BP – blood pressure, BM – body mass, CMC – Chris Moody Centre

* Downhill run occurring at Park campus or Chris Moody Centre

Data analysis:

All data was analysed using SPSS Version 26. Data was tested for normal distribution by the Shapiro-Wilk test. Significance levels were set at 0.05. A repeated measures Analysis of Variance was applied to assess the variability of the first three baseline assessments of muscle torque, with the co-efficient of variation determined by (standard deviation/mean) x 100. A paired samples t test was used to assess the difference of muscle soreness and torque from pre to post-EIMD protocol.

Results

Variability of first three maximal muscle torque assessments:

Due to abnormally high baseline muscle soreness measures on the first session, the first three muscle torque measures from two participants were excluded. Figure 3.4.1 displays the muscle torques for the first three baseline assessments. There was no difference amongst the baseline muscle torque scores (mean ± S.D 254.7 ± 102.2 Nm, 257.7 ± 101.6 Nm, 266.3 ± 111.1 Nm; effect of time, p=0.43) and pairwise comparisons also reveal no differences between any of the time points. The mean

within subject co-efficient of variance (CV) for the second and third muscle torques was 5.3%.

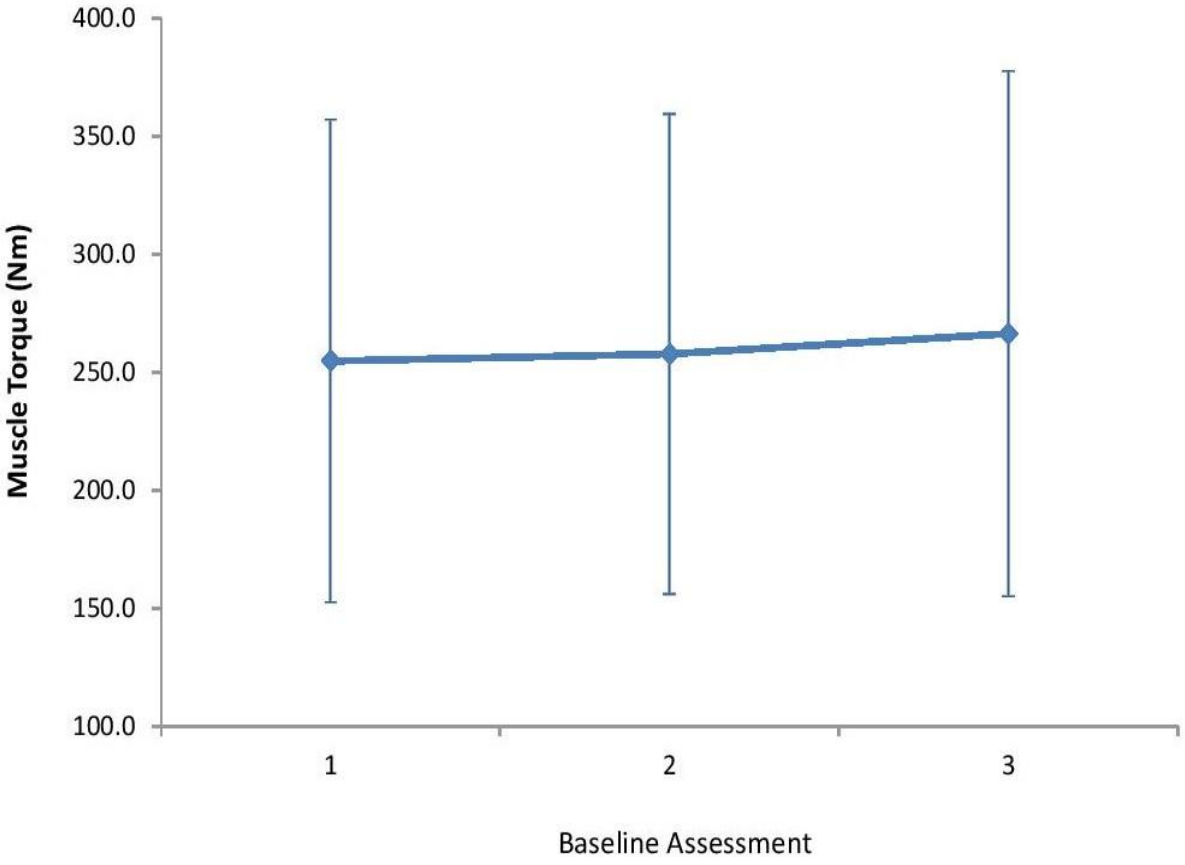


Figure 3.4.1: Variability of first three muscle torque assessments each separated by 24 hours. Data presented as means ± standard deviations. N=10.

Muscle damage markers pre and post-EIMD exercise protocol:

Figure 3.4.2 reveals the muscle torque outcomes pre and 24 hours post-downhill run at Park campus and Chris Moody Centre. There was no significant difference in maximal muscle torque between pre and post-downhill run at Park campus (266.7 ± 110.0 Nm vs. 258.6 ± 117.4 Nm, p=0.29) or Chris Moody Centre (260.1 ± 99.6 Nm vs. 268.7 ± 110.7 Nm, p=0.24).

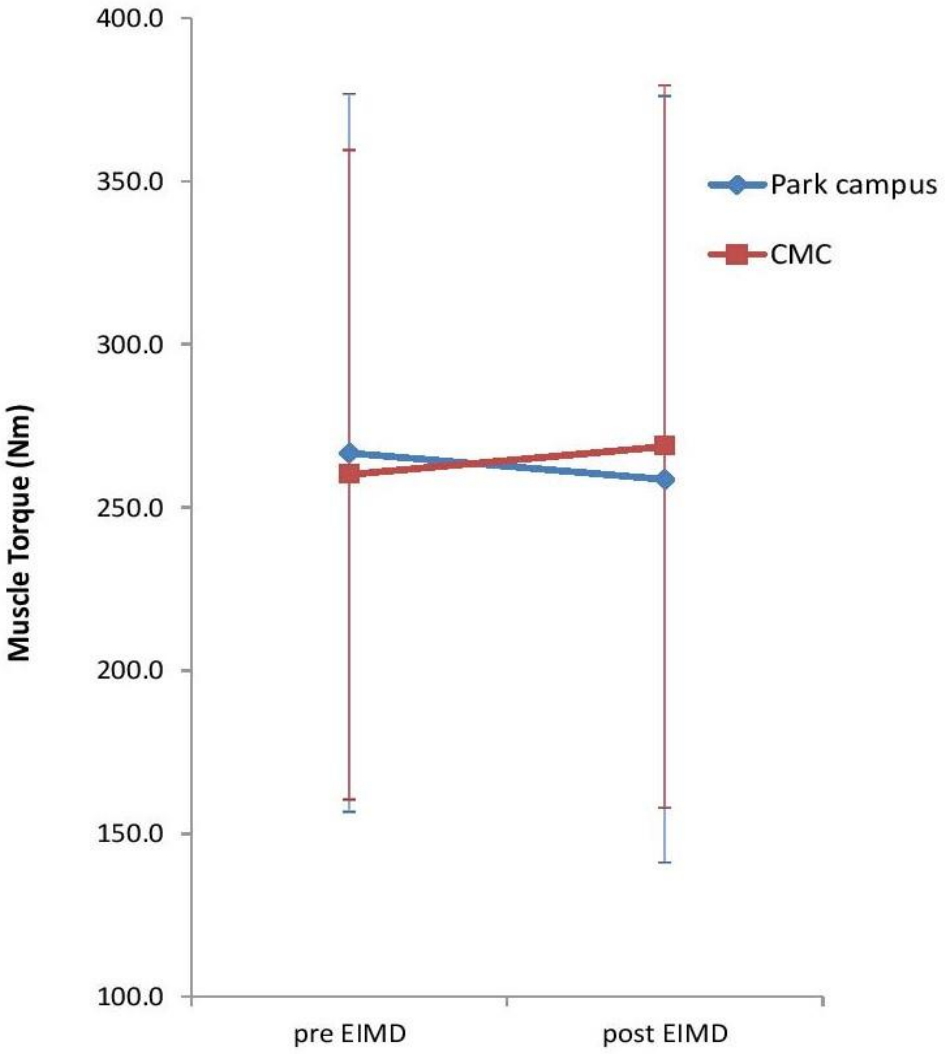


Figure 3.4.2: Muscle torque pre vs. post-downhill run. Data presented as means ± standard deviations. N=12.

Figure 3.4.3 displays the results for muscle soreness as measured by a visual analogue scale pre and 24 hours post-downhill run at Park campus and the Chris Moody Centre. A significant increase in muscle soreness was reported after the Park campus treadmill run (10% ± 12.6 vs. 34% ± 25.2, $p<0.01$). There was no difference in muscle soreness for the Chris Moody Centre trial (20% ± 20.4 vs. 18% ± 15.2, $p=0.57$).

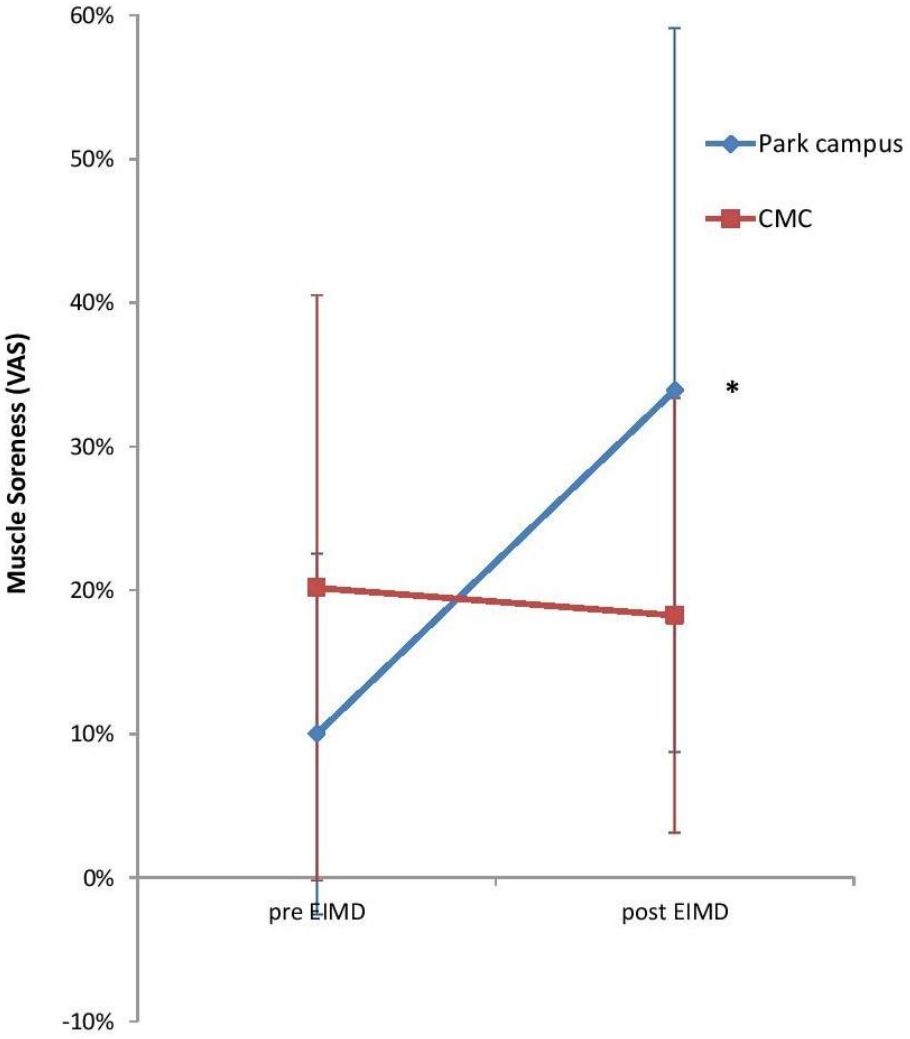


Figure 3.4.3: Muscle soreness pre vs. post-downhill run. Data presented as means ± standard deviations. N=12. *p<0.01 for increase in Park campus trial.

Discussion

The data presented above indicates that: 1) neither downhill running protocol was sufficient for inducing muscle damage, although the Park campus run caused muscle soreness; 2) participants became familiarised to the muscle torque assessments since there was no significant difference between the first three baseline measures.

One aim of this pilot study was to determine the reliability of maximal muscle torque assessments using a dynamometer. Since there was no difference between the first three baseline muscle torques, it can be inferred that just one familiarisation session

would be appropriate in the further studies. In the context of the ensuing studies in the body of this work, it is expected that the initial familiarisation session will not significantly impact the muscle torque assessment before the cryotherapy intervention. Furthermore, the average within subject co-efficient of variance was 5.3% excluding the initial familiarisation session. This indicates the day to day variance for a given subject and demonstrates that the resting muscle torque assessments on the Biodex were consistent and little or no learning effect occurred. Thus, one familiarisation session of muscle torque shall be sufficient for the subsequent studies.

Numerous studies have successfully demonstrated that downhill running causes EIMD (Fortes *et al.,* 2013; Malm *et al.,* 2004). In this pilot study, the downhill running protocols at both testing sites were insufficient to cause EIMD based on the lack of impact on muscle torques. Since the downhill running bout at Park campus caused a significant rise in muscle soreness, the eccentric contractions that occurred during this downhill run were causing some level of disruption to the muscle fibres. The lack of significant decrease in muscle torque demonstrates that there was little impact on the functional capacity of the quadriceps. Thus there is disparity between these two markers of muscle damage.

It is probable that the duration of the run was too short. A duration of 20 minutes was trialled in order to minimise discomfort to participants, as well as potential risk of drop outs and still determine if this run could be used appropriately in the main studies. Previous research studies that have used downhill running to examine muscle damage implemented durations of 30 minutes (Chen *et al.,* 2007), 45 minutes (Malm *et al.,* 2004) and 60 minutes (Dolci *et al.,* 2015). It is reasonable to suggest that increasing the length of the downhill run at Park campus would cause more disruption to the muscle fibres and thus more significant decrements in muscle torque. Exercise intensity, whilst being a potential factor, was set at 70% of estimated maximum heart rate which does not appear to deviate much from the intensities used in other downhill running protocols (60-70% VO_2 max). With regards to the decline gradient, 10% has commonly been used previously (Byrnes *et al.,* 1985; Dolci *et al.,* 2015; Fortes *et al.,* 2013) with the steepest gradient in the literature being 15% (Chen *et al.,* 2007). Increasing the decline beyond 10% could be uncomfortable and unsafe for participants. The 3% decline treadmill run at the Chris Moody Centre was clearly

not appropriate as a muscle damaging exercise bout since there was no impact on muscle torque or muscle soreness.

Another factor to consider for subsequent studies is the fitness levels and training status of participants. Some of the participants recruited for this study were trained athletes who may have been accustomed to muscle damaging exercises as part of their training. It is possible that short downhill running bouts would not cause significant decrements in muscle torque for this reason. For subsequent studies it would be beneficial to include a variety of subjects of different fitness levels.

In conclusion, the downhill running protocol at Park campus was only partially successful in inducing muscular damage, but it is conceivable that increasing the duration and/or intensity of this bout would make the protocol appropriate for the main studies to follow. Additionally, one familiarisation session for the dynamometer appears to be sufficient.

3.5. Pilot Study 2 – Creatine Kinase variability

Aims

The purpose of the second pilot study was three-fold:

- Firstly, to assess the reliability of blood creatine kinase (CK) measures using a Retroflon blood analyser and CK test strips as a secondary marker of muscle damage. There is also usually large variation in plasma CK response within participants owing to the different isoforms of CK as well as the specific nature of previous exercise bouts.
- Secondly, it was beneficial to establish the feasibility of acquiring whole blood samples post-WBC treatment, due to the induced 'vascular peripheral shutdown' (Selfe *et al.,* 2014) reported after WBC treatment. Should the possibility of acquiring blood samples post-WBC prove difficult, variations in body posture post-cryotherapy treatments (e.g. drawing blood whilst participants are in the supine position) or obtaining fingerstick blood samples as opposed to venepuncture shall be considered for the main studies.

- A final purpose of this pilot study was to establish familiarity with analysing CK levels using the Retroflon blood analyser.

Methods

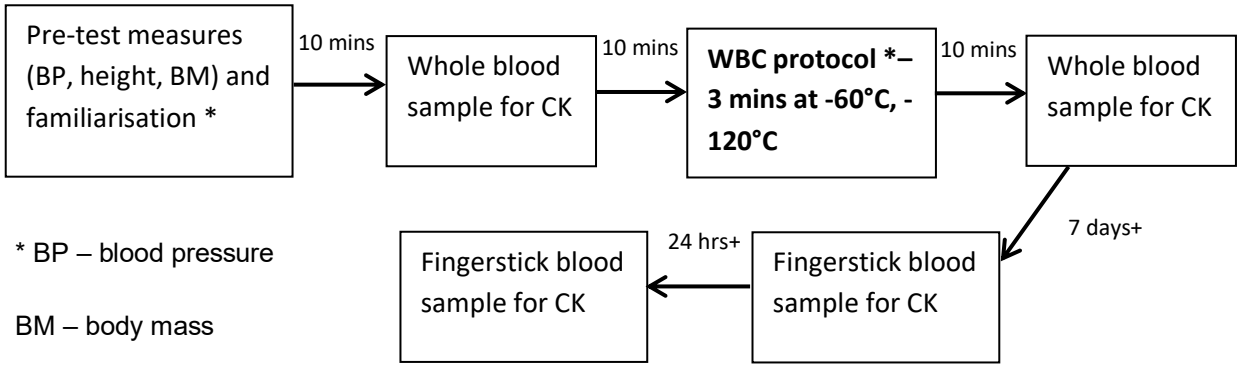
Participants:

Ten healthy male participants aged 18 or over (mean ± SD age 35.8 ± 18.3 years, height 1.78 ± 0.1m, body mass 75.6 ± 9.3 kg, body mass index 24.0 ± 2.4 kg/m^2) were recruited for this pilot study. All were required to be free from conditions (Appendix 2) that would exclude them from whole body cryotherapy treatment. These were determined through a medical questionnaire (Appendix 3) and pre-testing measures. Participants were asked to refrain from strenuous exercise and alcohol 24 hours prior to each session.

Protocol:

Participants were familiarised with the cryogenic chamber and procedures. Participants were then asked to report to the centre three times for testing. During the initial visit to the laboratory, baseline measures including resting systolic and diastolic blood pressure, body mass and height were recorded as outlined in sections 3.1 and 3.2. Participants who were found to be hypertensive (blood pressure exceeding 140/90 mm/Hg) were excluded from the study. Ten minutes following baseline measures, a blood sample was drawn via venepuncture whilst in a resting state and seated position. Participants then underwent cryotherapy treatment 10 minutes post-blood sample collection with the protocol as described in section 3.3. A second blood sample was obtained 10 minutes post-cryotherapy. After a week, participants returned to the laboratory twice on separate days (separated by at least 24 hours) to provide resting fingerstick samples.

Schematic for pilot study 2:



Blood Handling:

Fingerstick blood sampling was used to assess CK as described in section 3.2. For the secondary purpose of determining feasibility of obtaining samples pre and post-WBC, whole body samples were obtained by venepuncture into 6ml EDTA vacutainer tubes. Plasma creatine kinase was assessed as described in section 3.2.

Data analysis:

All data was analysed using SPSS Version 26. Data was tested for normal distribution by the Shapiro-Wilk test. Significance levels were set at 0.05. The co-efficient of variation for the two baseline blood CK samples was calculated by (standard deviation/mean) x 100. A paired samples t test was applied to assess the difference in plasma CK pre and post-WBC.

Results

The plasma CK results are displayed in Figures 3.5.1 and 3.5.2 for whole blood samples and fingerstick samples respectively. A paired t test revealed no significant increase in plasma CK pre to post-WBC treatment (124.0 ± 53.0 IU/L vs. 141.3 ± 53.5 IU/L, p=0.12), however blood samples from four of the participants post-WBC was not acquired. For the fingerstick samples, there was no difference between the two samples (148.6 ± 85.5 IU/L vs. 140.7 ± 83.0 IU/L, p=0.43). The mean subject co-efficient of variance for the two baseline fingerstick measures was 11.7%.

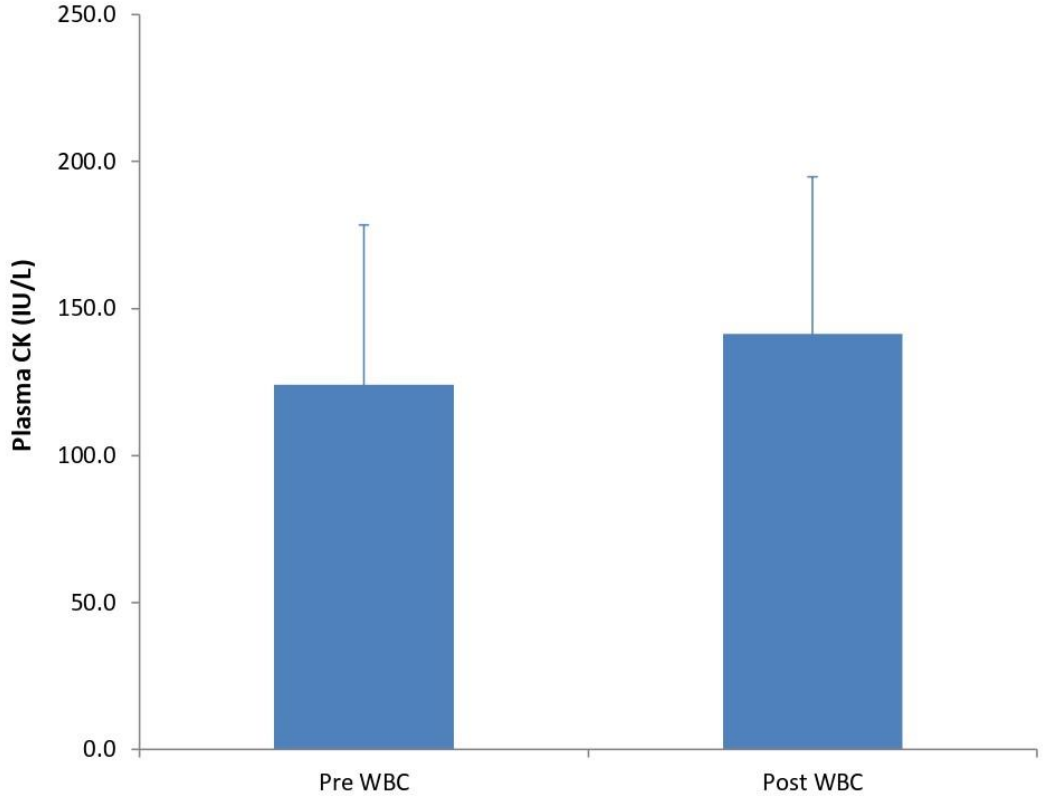


Figure 3.5.1: Plasma CK response pre and post-WBC. Data presented as means + standard deviations. N=6.

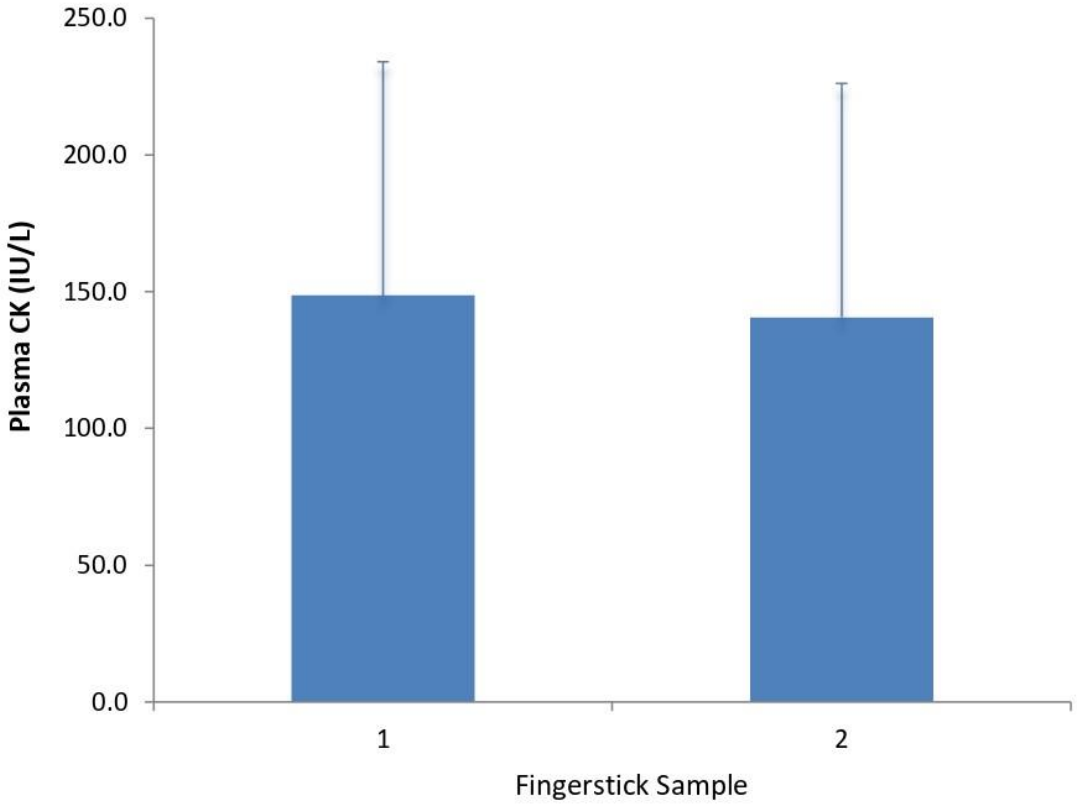


Figure 3.5.2: Fingerstick blood CK samples for variability. Data presented as means + standard deviations N=8

Discussion

In conclusion, there was initially slight difficultly obtaining blood samples post-WBC, potentially due to peripheral vascular shutdown. Furthermore, the fingerstick samples reveal a moderately high reliability due to low resting CK variability within individuals. Other studies have however reported lower intra-assay CVs of below 4% for CK (Horder *et al.,* 1991). This discrepancy could be due to different isoforms of CK and the inconsistent time period between participants' first and second samples (e.g. ranging from 24 to 96 hours). Methodological considerations ahead of future WBC studies include the time period of blood withdrawal post-cryotherapy. Clearly, fingerstick blood sampling with immediate assessment of creatine kinase is a preferable method for measuring this variable. It would therefore be prudent to consider the use of fingerstick blood samples as an alternative to venepuncture.

3.6. Pilot Study 3 – Alternative Muscle Damage protocol

Aims

The third pilot study aimed to evaluate the effectiveness and appropriateness of two potential EIMD exercise protocols. As the downhill running protocol in the first pilot study was insufficient to induce substantial muscle damage, it was necessary to determine if a prolonged treadmill protocol, resistance and/or plyometric exercises would be more appropriate. Muscle damage markers (maximal muscle torque and muscle soreness) were assessed pre and post-exercise and compared between downhill running and resistance exercises. Resistance exercises have been used (Roberts *et al.,* 2014) as well as plyometric exercises (Vieira *et al.,* 2016) as effective muscle damaging exercise models to assess recovery. Decrements in muscle torque were considered the predominant gauge for the success of the muscle damage protocol, with muscle soreness as a secondary measure. The main purpose of this pilot study was to determine whether a revised treadmill protocol and a resistance exercise protocol can induce sufficient muscle damage to cause significant changes to muscle torque and muscle soreness post-exercise. If this was the case, it was necessary to determine which protocol may be more appropriate to assess recovery from EIMD, predominantly by the extent of muscle torque loss.

Methods

Participants:

Eight healthy male participants aged 18 or over (mean ± SD age 29.5 ± 17.8 years, height 1.75 ± 0.1m, body mass 80.1 ± 13.5 kg, body mass index 26.0 ± 3.0 kg/m^2) were recruited and were free from cardiovascular or neural conditions. Participants' suitability to take part was determined via resting blood pressure measures as well as completion of a health questionnaire.

Protocol:

For each participant's first visit, baseline measures including resting blood pressure, body mass and height were recorded as specified in sections 3.1 and 3.2. Participants were familiarised to the upcoming muscle torque assessment with the setup being the same as pilot study 1. In order to determine one repetition maximums (1RM) for exercises to be performed during the resistance exercise protocol, participants were required to perform maximal contractions for three exercises: dumbbell lunge, barbell back squat and knee extension, with the procedures described in section 3.3.

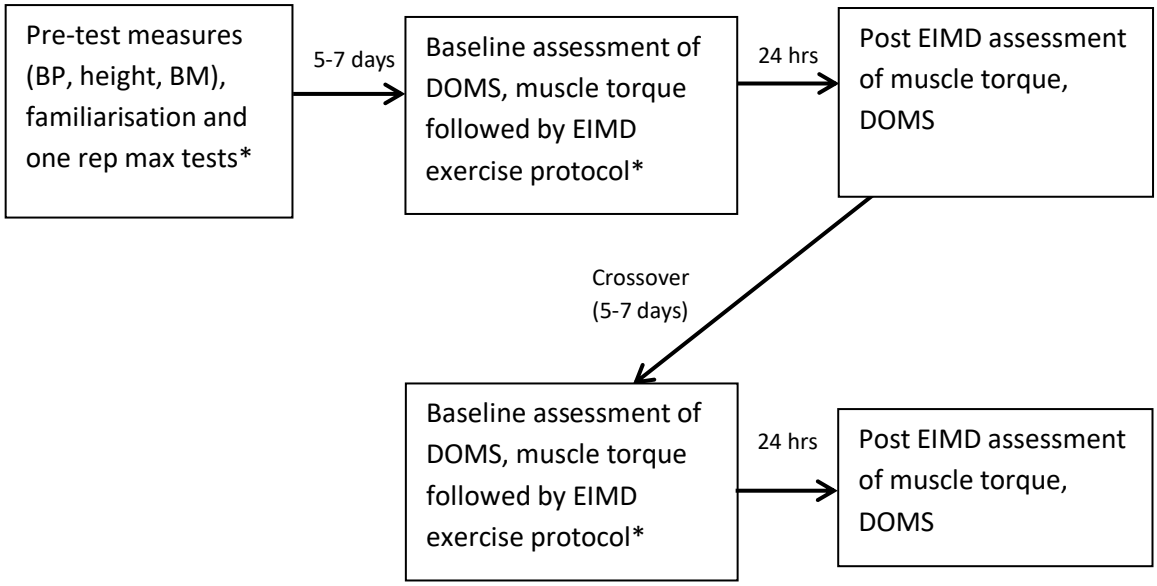
Baseline measures were then obtained for muscle damage markers 5 to 7 days later – muscle soreness and muscle torque. Subjective muscle soreness was assessed via a visual analogue scale (Gould *et al.,* 2001), as described in section 3.2, except this was measured twice. The first measurement involved participants squatting with their back against a wall (as in pilot study 1). The second assessment involved participants lying in a supine position with their legs extended. They maintained the position for three seconds and indicated the degree of pain felt by marking a distance on a separate scale to the one used for the wall squat assessment. The first pilot study (section 3.4) revealed that some participants reported mild muscle soreness for the assessment possibly due to the slight amount of strain imposed whilst holding the squat position. Therefore, a second assessment was used in this study to moderate the muscle soreness score, with a mean of the two measures recorded for analysis purposes. The muscle torque procedure was identical to the first pilot study, with the torque determined as the peak from four maximal contractions. Participants

underwent two exercise interventions in a randomised crossover design - downhill running and resistance based exercises, occurring 10 minutes after the preceding baseline measures. The downhill running procedure involved 30 minutes running at a 10% decline (Park campus) and intensity of 70% of estimated maximal heart rate, with heart rate and RPE monitored every 5 minutes. The resistance exercise protocol included movements chosen (section 3.3) to induce metabolic and mechanical damage on the muscle fibres.

Participants had their muscle damage markers re-assessed 24 hours after completion of the exercise protocol. Subjective muscle soreness was again assessed in duplicate via visual analogue scales. Muscle torque was determined as the maximal torque elicited from four maximal contractions.

Five to 7 days later, participants repeated the procedure for the different exercise protocol. Initial baseline measures of muscle soreness and maximal muscle torque were again taken 10 minutes prior to the exercise protocol. Twenty four hours after completion of this exercise protocol, participants underwent the muscle damage assessments as described prior. Responses were assessed pre and post exercise and compared between downhill running and resistance exercises. Participants were asked to refrain from alcohol and strenuous physical activity for 24 and 48 hours respectively prior to all testing sessions.

Schematic of sessions for pilot study 3:



* NB: The one repetition maximums were performed for three exercises: weighted lunges, barbell squat and leg extension.

* EIMD exercise protocol entailed 30 minutes downhill run or 13 sets of resistance exercises.

BP – blood pressure, BM – body mass, DOMS – delayed onset muscle soreness, EIMD – exercise-induced muscle damage

Data analysis:

All data was analysed using SPSS Version 26. Data was tested for normal distribution by the Shapiro-Wilk test. Significance levels were set at 0.05. A two way repeated measures analysis of variance was applied to assess muscle damage marker response across two time points – pre and post-EIMD protocol and comparing between the two exercise interventions. The difference in muscle damage markers (e.g. muscle torque) pre to post EIMD was compared between the two exercise conditions via a paired samples t test.

Results

Figures 3.6.1 displays the peak muscle torque response for the two exercise protocols. There was a slight decrease in muscle torque pre to 24 hours post-downhill run which approached significance (230.1 ± 73.8 Nm vs. 214.3 ± 62.8 Nm, p=0.07). There was no decrease in muscle torque pre to post-resistance exercises (208.4 ± 61.9 Nm vs. 206.4 ± 59.6 Nm, p=0.87). The interaction effect between trial and time was non-significant (p=0.35).

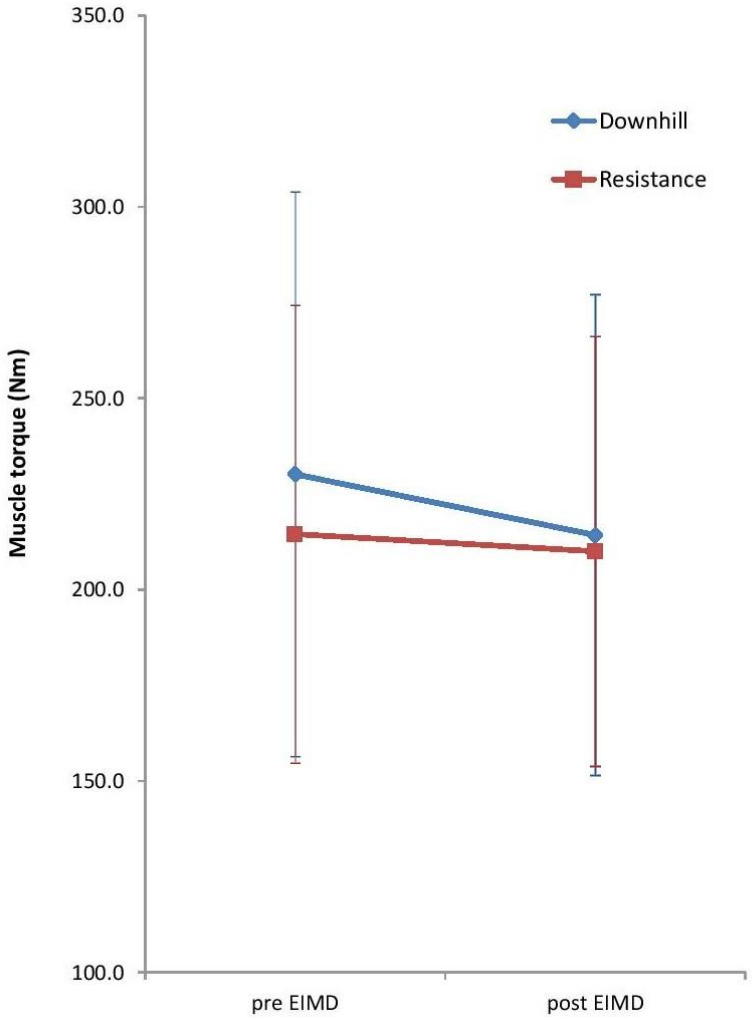


Figure 3.6.1: Muscle torque pre vs. post exercise-induced muscle damage (EIMD) exercise protocol. Standard deviations indicated by error bars. N=8

Figure 3.6.2 displays the muscle soreness response for the two trials, as measured by the mean of the two visual analogue scales. There was a significant increase in

muscle soreness for both trials (3% vs. 15% for downhill run, 3% vs. 15% for resistance exercises, p=0.01 for both)

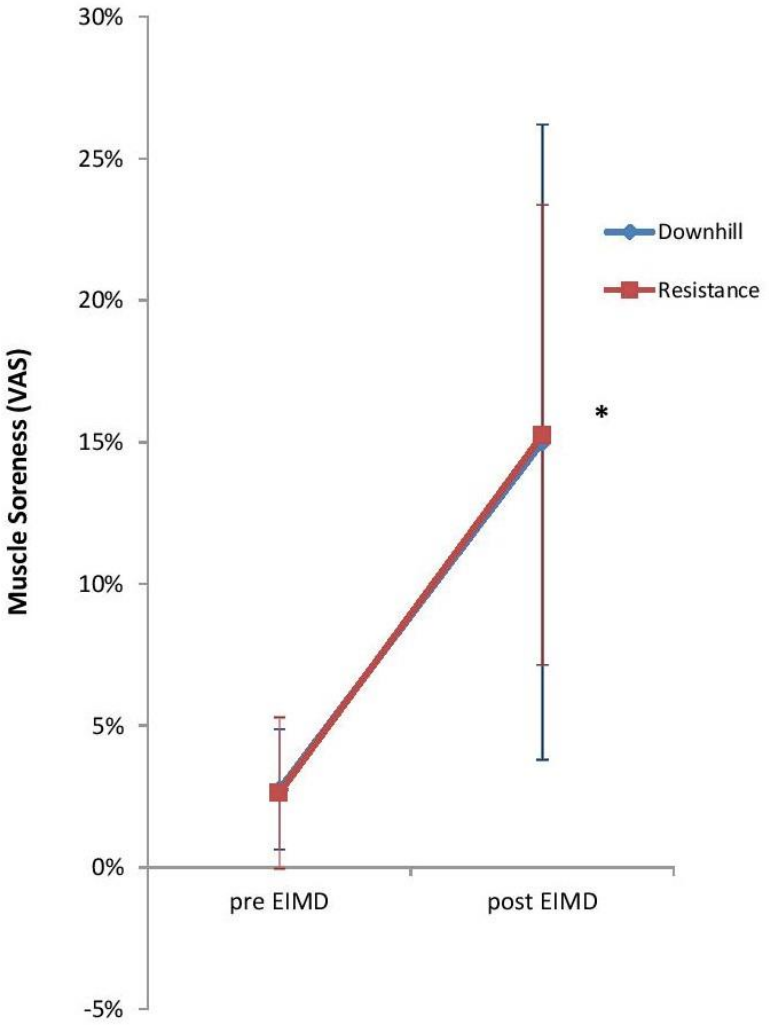


Figure 3.6.2: Muscle soreness pre vs post exercise-induced muscle damage (EIMD) protocol. Standard deviations indicated by error bars. *p=0.01 for increase in both trials. N=8

Discussion

The predominant finding from this pilot study was that the revised treadmill run at PC induced slight muscle damage, due to the nearly significant decrease (p=0.07) in muscle torque and significant increase in muscle soreness. Despite significant muscle soreness also being reported for the resistance exercise routine, there was no impact on muscle torque. This suggests that the downhill run was more appropriate as a muscle damaging exercise than the resistance exercises due to the more substantial impact on muscle torque decrement. However, since statistical

significance was not attained, it was deemed appropriate to reconsider the exercise protocol for subsequent studies assessing WBC for post-exercise recovery.

One notable difference in the methodology between the first and third pilot studies was the inclusion of a second muscle soreness scale. Lying in a supine position places the legs under less strain than squatting against a wall, thus lower soreness measures are likely to be reported. Therefore, the risk of excluding participants on the premise of reporting soreness was reduced. In addition, participants were asked to avoid all strenuous activity 48 hours prior to all testing sessions (as opposed to 24 hours in the first pilot study) to ensure minimal reporting of muscle soreness before the exercise bouts.

It should also be noted that the muscle torque losses in this study were relatively modest compared to previous studies (Ferreira-Junior *et al.,* 2015; Nelson *et al.,* 2004). The small sample size in this pilot study may be an explanation for the relative lack of muscle damage severity.

In conclusion, the 30 minute downhill run at Park campus caused mild muscle damage and would be more appropriate to use as a muscle damaging exercise bout than the protocol used in pilot study 1. However, since statistical significance was still not attained in muscle torque loss, a revised downhill running protocol should therefore be considered (section 3.7). The resistance exercise routine was not effective in causing muscle damage, despite inducing muscle soreness.

3.7 Pilot Study 3 re-trial

Aims

The principal aim of the re-trial of the third pilot study was to establish a downhill run model that would cause a statistically significant decrease in muscle torque 24 hours post-exercise. Apart from intensity, the only variable yet to be experimented was the gradient. Thus, a downhill run of 30 minutes duration, 15% decline and 70% of estimated maximum HR was trialled to determine whether significant muscle damage would be induced, therefore establishing its suitability as an exercise protocol for the following main studies.

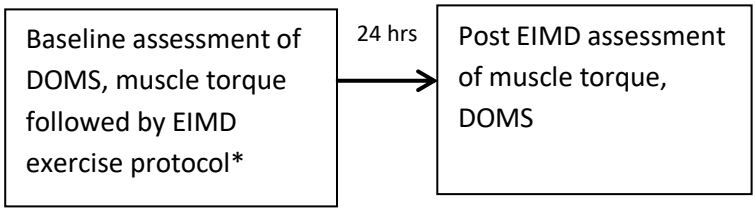
Methods

Participants:

Five healthy participants took part in the re-trial (three male, two female, aged 18-27). All were accustomed to exercise on a regular basis and were free from cardiovascular or neural conditions.

Protocol:

The protocol was very similar to that of pilot study 3, except the steeper downhill run was used as a stand-alone muscle damaging exercise protocol in a simple pre-test post-test design. Participants arrived to the laboratory for the first session, with the same protocol as the second session for the third pilot study (section 3.6), except the 30 minute downhill run was performed at a decline gradient of 15%. A familiarisation session for this re-trial was not deemed necessary due to the lack of difference between familiarisation torques and actual torques in the previous pilot studies (data not shown) and low number of measures in this short study. Muscle torques and muscle soreness were assessed as in pilot study 1 with post measures occurring 24 hours after the exercise bout.



* EIMD exercise protocol entailed 30 minutes downhill run at 15% gradient.

Data analysis:

All data was analysed using SPSS Version 26. Data was tested for normal distribution by the Shapiro-Wilk test. Significance levels were set at 0.05. The difference in muscle damage markers (e.g. muscle torque) pre to post-EIMD was compared via a paired samples t test.

Results

Figures 3.7.1 and 3.7.2 display the responses for muscle torque and muscle soreness respectively for the new treadmill protocol. There was a significant decrease in muscle torque pre to 24 hours post-downhill run (216.4 ± 47.5 Nm vs. 173.4 ± 49.2 Nm, p=0.03). There was also a significant increase in muscle soreness (11% ± 14.1 vs. 42% ± 17.9, p=0.03).

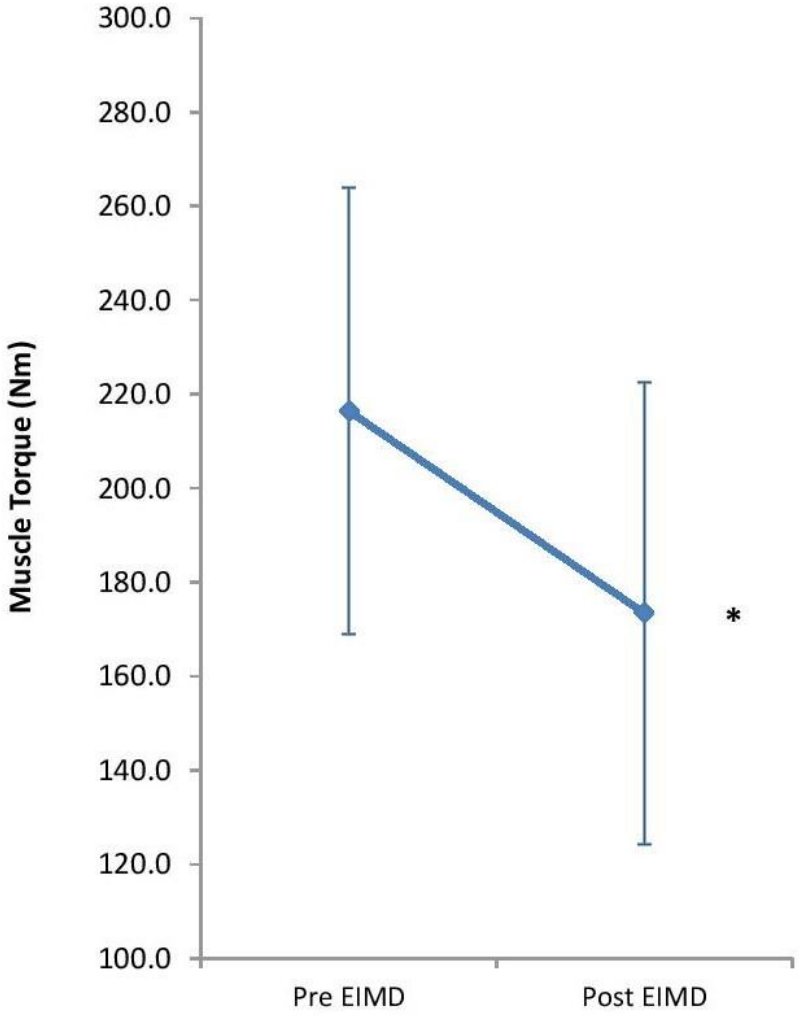


Figure 3.7.1: Muscle torque pre vs post exercise-induced muscle damage (EIMD) protocol. Standard deviations indicated by error bars. *p<0.05. N=5

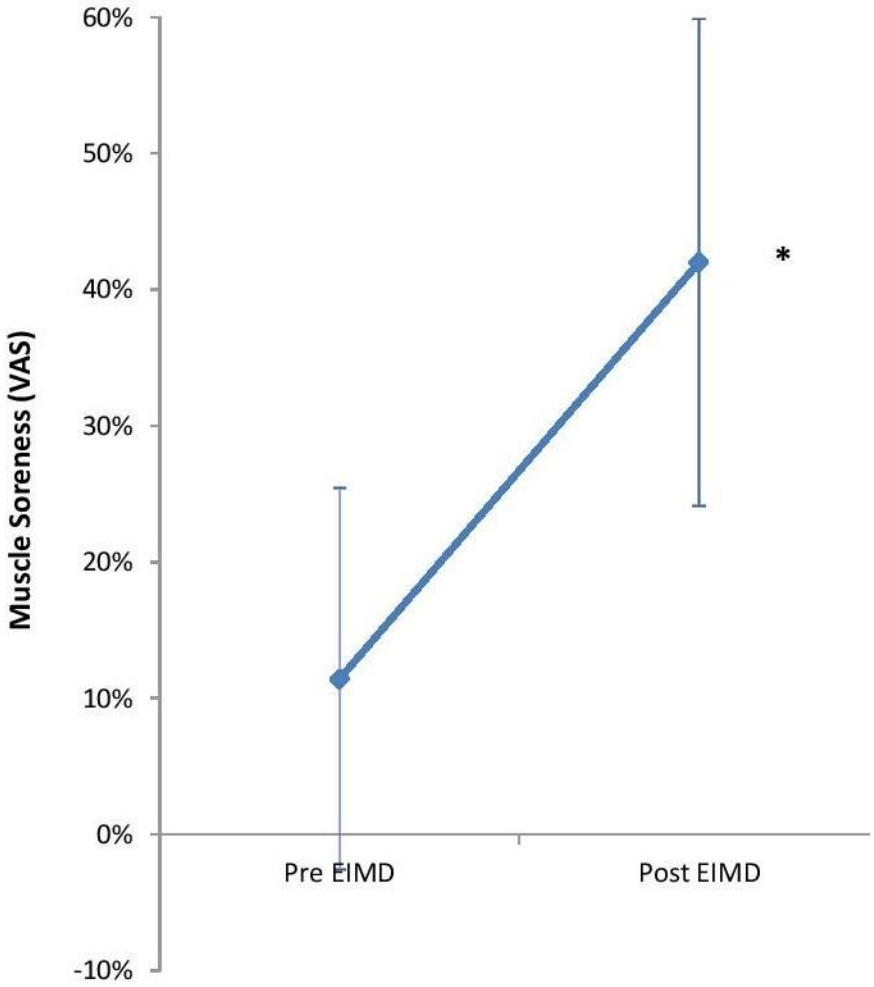


Figure 3.7.2: Muscle soreness pre vs post exercise-induced muscle damage (EIMD) protocol. Standard deviations indicated by error bars. *p<0.05. N=5

Discussion

The revised treadmill protocol for the third pilot study caused substantial muscle damage due to the significant decrease in muscle torque as well as increase in muscle soreness. This confirms the suitability of this exercise model for subsequent studies in assessing the impact of whole body cryotherapy for post-exercise recovery. The steep decline of 15% also proved to be safe and reasonable since no adverse effects were reported by the participants other than the expected muscle soreness, and all participants successfully completed the protocol. This alleviated any previous concern of a 15% gradient being excessively steep for the safety and comfort of participants. Ultimately, a 30 minute downhill run at 15% decline achieved

an appropriate balance between maximising muscle damage and minimising discomfort, time and effort from the participants.

Despite 15% not being a common downhill gradient used in the literature, it was deemed necessary to trial this gradient since duration had already been manipulated. It was essential for substantial muscle damage to occur since any assessment of post-exercise recovery would otherwise be invalid. Furthermore, statistical significance was attained with only 5 participants, so the extent of muscle damage was clearly more magnified than the previously trialled protocols. Whilst the initially attempted 30 minute downhill run in the third pilot study (10% decline) caused some degree of muscle torque loss, a type 2 error may have been caused by a lack of statistical significance – i.e. possible lack of power due to small sample size. However, it was considered more appropriate to experiment with a new exercise model instead of testing more participants on the 10% run. In addition, a type 1 error is unlikely in the 15% decline re-trial due to the expected occurrence of muscle soreness, which was more profound than following the 10% decline (Figure 3.4.3).

No studies to date have examined whole body cryotherapy responses following downhill running. With a clear impact of downhill running on muscle function, it remains of interest to determine whether WBC can restore muscle function and general homeostasis that has been perturbed in this way. This is intended to be a novel aspect for this body of research (chapter 4).

In conclusion, an appropriate muscle damaging exercise model was established. A 30 minute downhill run at a gradient of 15% shall be utilised to assess whole body cryotherapy for post-exercise in further studies (chapters 4 and 5).

Table 3.7.1: Summary of exercise protocols trialled in pilot studies and impact on muscle torque 24 hours post.

Exercise protocol	Impact on muscle torque	Significance value for decrease in muscle torque pre to post downhill run	Suitability for use in main studies
Resistance exercise routine – 13 sets of lower body exercises	No difference. 208.4Nm vs. 206.4Nm	p=0.87	No
Downhill run – 20 mins, 3% decline	No difference. 260.1Nm vs. 268.7Nm	n/a	No
Downhill run - 20 mins, 10% decline	No difference. 266.7Nm vs. 258.6Nm	p=0.29	No
Downhill run - 30 mins, 10% decline	Slight decrease. 230.1Nm vs. 214.3Nm	p=0.07	No
Downhill run - 30 mins, 15% decline	Significant decrease. 216.4Nm vs. 173.4Nm	p=0.03	Yes

CHAPTER 4 – MAIN STUDY 1

The Effects of Age and Body Fat Content on Post-Downhill Run Recovery following Whole Body Cryotherapy

4.1. Introduction

Whole Body Cryotherapy (WBC) is an extremely cold treatment (typically below -100°C) that has been used for pain remission, musculoskeletal disorders and skin lesions (Lubkowska, 2012; Straburzyńska-Lupa *et al.,* 2018). Its recent emergence in sport and exercise has added a unique perspective to sports recovery practice (Lombardi *et al.,* 2017; Partridge *et al.,* 2019). As reported in chapter 2, the extensive and diverse reported effects of WBC post-exercise include attenuated blood markers such as creatine kinase (CK) (Ziemann *et al.,* 2012), muscle soreness (Fonda & Sarabon, 2013), inflammation (Pournot *et al.,* 2011; Ziemann *et al.,* 2013) and alleviated reductions in muscle torque (Ferreira-Junior *et al.,* 2015; Hausswirth *et al.,* 2011). Despite these effects, the precise impact of WBC in sports and performance remains equivocal (Krueger *et al*., 2018; Vieira *et al.,* 2015), whilst negative effects have also been reported (Wilson *et al.,* 2018). Consequently, there remains a need to address the efficacy of WBC in greater depth to better inform the sporting community of its overall merit.

A potentially important consideration when evaluating the impact of WBC treatment is the inter-individual variability. The factors of age and body fat content have already been discussed (section 2.6.5) and may be significant in informing optimal practices for the treatment. Probable causes of reduced recovery and performance capacity with ageing include muscle mass and strength loss (sarcopenia), oxidative damage and chronic inflammation (McCormick & Vasilaki, 2018), which could affect responses to cryotherapy post-exercise. There is a paucity of literature concerning differences in responses to cryotherapy between young and old populations. It is understood that the cutaneous vasoconstrictor and cold-induced vasodilation response following extreme cold can be blunted in older compared to younger individuals (Young *et al.,* 1996). Functional decline with ageing is evident, for instance a reduced responsiveness of the blood vessels (Knight & Nigam, 2007). This may be significant in the context of cryotherapy, because of the impact on blood redistribution and heat transfer. It is therefore conceivable that older individuals would respond to the treatment less optimally than their younger counterparts.

Outcomes to cryotherapy treatments among differing body compositions have not been investigated extensively despite body fat content influencing cold exposure reactions (Stephens *et al.,* 2018). Higher fat individuals may retain core and tissue

temperatures to larger extents following cryotherapy (owing to reduced vasodilation) compared to leaner individuals (Stocks *et al.,* 2004). Additionally, subcutaneous fat provides thermal insulation and decreases thermal conductance (Young *et al.,* 1996). Thus, the response and overall tolerance to cold temperatures can vary accordingly. A link has been demonstrated between body fat content and duration required to reduce intramuscular temperatures following cold treatments (Otte *et al.,* 2002), owing to the insulatory properties of adipose tissue. The theory that higher adiposity could affect the response to WBC is supported by observations of strong negative correlations between body fat percentage and skin temperatures (Cuttell *et al*., 2017; Hammond *et al.,* 2014). Despite the reported differences in responses to cold between different body compositions, the implications of such variances for WBC application post-exercise remains under-investigated.

This study aimed to examine the effect of WBC following a downhill run, a common exercise protocol imposing continual eccentric contractions on the quadriceps muscles. Such bouts cause muscle torque losses, elevated soreness, plasma CK, inflammatory cytokines and reduced running economy (Chen *et al*., 2009; Malm *et al.,* 2004; Peake *et al.,* 2005; Smith *et al*., 2007), established characteristics of exercise-induced muscle damage (EIMD). One theme of interest is how cryotherapy could potentially alleviate this muscle breakdown and damage, which would present an advantage for athletes since EIMD has negative consequences on locomotor biomechanics and subsequent performance (Assumpcao *et al.,* 2013; Cheung *et al.,* 2003). Since downhill running is a whole-body exercise that stresses several physiological systems, it is of interest to determine if WBC could enhance recovery following this modality. Other commonly adopted damage protocols, such as isolated eccentric leg extensions (Costello *et al*., 2012) and arm curls (Sayers & Clarkson, 2001; Yoon & Kim, 2020) may be less sports specific, so any WBC-induced responses may be less applicable for general sports recovery. Despite the extensive literature on downhill running and WBC, no study has yet to assess the impact of WBC on recovery following a downhill running bout. Additionally, previous studies that have demonstrated positive effects for WBC treating EIMD (e.g. Fonda & Sarabon, 2013; Hauswirth *et al.,* 2011) typically used multiple treatments post-exercise, which are less economical and practical than a single treatment.

Therefore, the principal aims of this study were as follows:

1. To assess the overall impact of a single WBC treatment on recovery following a downhill run.

2. To assess the impact of age and body fat content on recovery response to a single WBC treatment post-exercise.

It was hypothesised that 1) WBC would attenuate muscle damage markers post-downhill running, thereby supporting its use as a means to enhance recovery after muscle damaging exercise; and 2) younger men with lower body fat contents would respond more positively to WBC than other populations.

4.2. Methods

4.2.1. Participants

A sample size calculation (G*Power: significance level 0.05, power 0.8, effect size 0.5) revealed that 9 participants per group would be appropriate to detect an effect. Forty-one male volunteers (mean ± SD age 42.0 ± 13.7 years, height 1.76 ± 0.08m, body mass 75.2 ± 10.8 kg, body fat 19.2 ± 4.5%) were recruited for the study, which adopted an independent groups design. Participants were randomly assigned as cryotherapy (WBC, n=26) and control (CON, n=15). To assess the influence of differing ages and body fat contents, the WBC group was sub-divided into old (OLD, ≥45, mean ± SD age 58.1 ± 7.9 years, n=10) and young (YNG, <40, mean ± SD age 29.2 ± 7.6 years, n=13) (Baross *et al.,* 2013), as well as high fat (HFAT, ≥20%, mean ± SD body fat 23.0 ± 2.9%, n=10) and low fat (LFAT, ≤15%, mean ± SD 13.8 ± 1.4%, n=8) groups (Yoon & Kim, 2020). Three WBC participants were aged 40-44 and 8 WBC participants had 15.5-19.5% body fat, which were not part of these sub-groups. They were still included in the overall analysis between WBC and CON.

All participants were of a suitable fitness level for the demands of the study, consistently partaking in physical activity a minimum of twice a week. Prior to further screening and assessment, all participants' blood pressure was assessed and written informed consent was provided. Ethical approval was obtained from the University of Northampton Graduate School Research Ethics Committee.

Sample characteristics for each group are summarised in Table 4.2.1.

Table 4.2.1: Summary of characteristics for whole body cryotherapy (WBC) and control (CON) participants. Data presented as means ± standard deviations.

	WBC (n=26)	**CON (n=15)**	**OVERALL (n=41)**	**T test between WBC and CON**
Age (yrs)	41.8 ± 15.5	42.3 ± 10.4	42.0 ± 13.7	p=0.93
Height (m)	1.78 ± 0.09	1.75 ± 0.06	1.76 ± 0.08	p=0.21
Body mass (kg)	74.9 ± 10.8	75.6 ± 11.1	75.2 ± 10.8	p=0.85
Body mass index (kg/m^2)	23.7 ± 2.2	24.7 ± 2.9	24.1 ± 2.5	p=0.22
Body fat %	18.8 ± 4.3	20.0 ± 4.9	19.2 ± 4.5	p=0.4
Absolute VO_2 max (l/min)	3.61 ± 0.55	3.53 ± 0.63	3.58 ± 0.57	p=0.67
Relative VO_2 max (ml/min/kg)	48.4 ± 5.1	46.8 ± 6.5	47.8 ± 5.6	p=0.32

4.2.2. Initial Trial

Participants were asked to refrain from alcohol and strenuous exercise for 24 and 48 hours respectively prior to all trials. Initially, participants' anthropometric characteristics were assessed, including height and body mass. Body fat content was assessed by skin-fold calipers, as explained previously (section 3.2).

Participants were familiarised to a muscle torque assessment using the Biodex dynamometer. This involved two submaximal isometric contractions (60% and 80% effort), followed by a singular maximal contraction, as explained previously (section 3.2). All contractions were 5 seconds in duration.

Participants prepared for their maximal aerobic capacity (VO_2 max) assessment on the treadmill. VO_2 max was measured using an online breath by breath analyser following an incremental treadmill protocol as explained in section 3.2. Following exhaustion and test completion of the test, participants were allowed fluids and a

brief cool down period. The absolute and relative VO_2 max values were reported and 60% of the absolute VO_2 max was calculated.

Participants then completed the muscle torque assessment on the Biodex dynamometer, involving four maximal contractions as explained previously (section 3.2). The best torque produced from the four contractions was determined as the individuals pre-torque score. Participants were then allowed to leave the laboratory.

4.2.3. Main Trial

Within 3 to 14 days of the first trial, participants returned to the laboratory (ambient temperature 20 ± 0.5°C) to perform their main trial. Initially, skin temperatures were assessed using four sites as described previously (section 3.2). Tympanic temperature was also assessed. Participants then provided a 30µl fingerstick blood sample for the measurement of their creatine kinase (CK) and haemoglobin levels. Muscle soreness, thermal comfort and mental wellbeing were assessed via VAS scales as described previously.

Participants commenced their 30 minute downhill run at 15% gradient following a two minute walk period at 5 km/h to adjust to the gradient. The treadmill speed was gradually adjusted as described previously (section 3.3) with HR and RPE monitored at regular intervals. The predetermined HR was extrapolated from the VO_2 max vs. HR relationship, so that a running intensity corresponding to 60% of their absolute VO_2 max was maintained. After completing the run, participants dried themselves and were provided fluids *ad libitum*. Tympanic and skin temperatures were immediately assessed as described previously, followed by CK, haemoglobin, muscle soreness, thermal comfort and mental wellbeing.

Cryotherapy participants were then transported to the Chris Moody Centre for their WBC treatment at -120°C (procedure explained in section 3.3) scheduled for 60 minutes post-downhill run. Control participants remained seated in the laboratory under controlled conditions (20°C). Immediately after the 3 minute WBC treatment, participants removed their cryotherapy gear and placed their usual footwear on. Thermal images were again taken 5 minutes post-WBC. Tympanic temperature, CK, haemoglobin and VAS scales for muscle soreness, thermal comfort and mental wellbeing were measured 10 minutes post-WBC as described previously. Heart rate

was also measured before participants were allowed to leave the centre. Measures for the control participants were recorded at the same time points as stated above.

4.2.4. Final Trial

Participants returned to the laboratory 24 hours post-downhill run for their final assessments. Blood CK, haemoglobin, muscle soreness and wellbeing were measured as described previously. Participants then underwent their post muscle torque assessment, following the same protocol as their first trial. The peak torque was determined as the highest of four maximal contractions.

The study protocol is summarised in Figure 4.2.1.

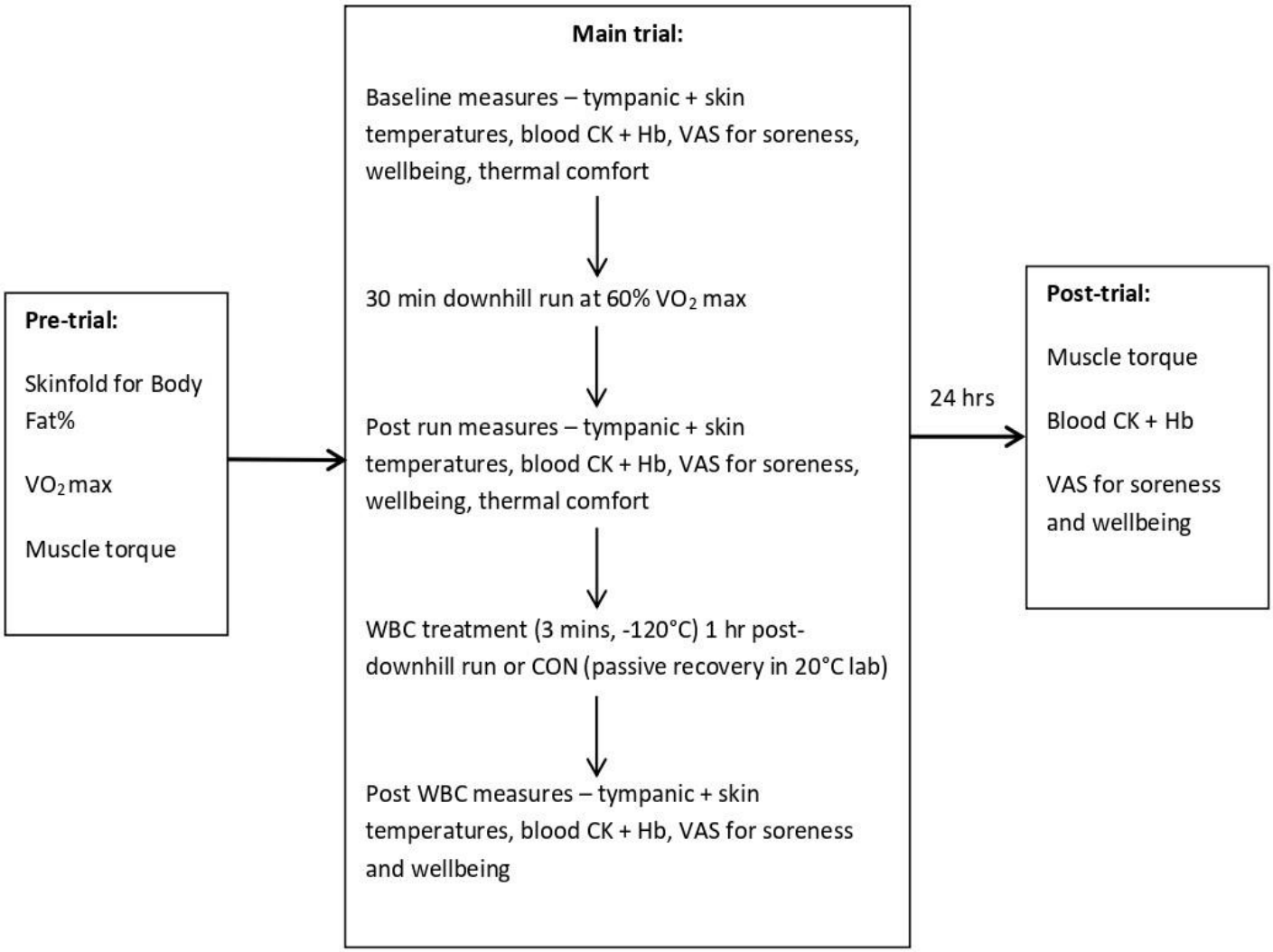


Figure 4.2.1: Protocol summary of measures for each trial in main study 1. WBC – Whole Body Cryotherapy; CON – Control; CK – creatine kinase; Hb – Haemoglobin; VAS – visual analogue scale; HR – heart rate.

4.2.5. Statistical Analyses

All data was analysed using SPSS Version 26. Data for all variables was assessed for normal distribution by the Shapiro-Wilk test and extent of skewness and kurtosis. Muscle torque, soreness, CK and VAS significantly deviated from normality and were therefore log or square root transformed as appropriate. A two-way repeated measures ANOVA was used to assess the interaction effect between group (WBC vs. CON; OLD vs. YNG; HFAT vs. LFAT) and time for all major dependent variables. Paired t tests and pairwise comparisons with a Bonferroni correction were applied where necessary to examine differences between specific timepoints. Effect sizes (Cohen's d) and 95% confidence intervals (CI) were calculated for muscle torque, the main dependent variable of interest. Significance levels were set at 0.05.

4.3. Results

WBC vs CON

Muscle Torque

There was no significant difference in pre-muscle torque between WBC and CON groups (p=0.27). There was a significant decrease in maximal isometric muscle torque for both groups following the downhill run (WBC, 220.6 ± 61.4 Nm, 95% CI [197.0, 244.2] vs. 208.3 ± 67.6 Nm, 95% CI [182.4, 234.3], p=0.02, d=0.19; CON, 239.7 ± 51.1 Nm, 95% CI [213.8, 265.5] vs. 212.1 ± 46.3 Nm, 95% CI [188.7, 235.6], p<0.01, d=0.57, Figure 4.3.1). The mean decreases were 12.2 ± 24.8 Nm (6.4%) and 27.5 ± 14.6 Nm (11.5%) for WBC and CON respectively with a significant difference between groups (p=0.04, d=0.67). The overall difference between groups over time was non-significant (interaction effect, p=0.10).

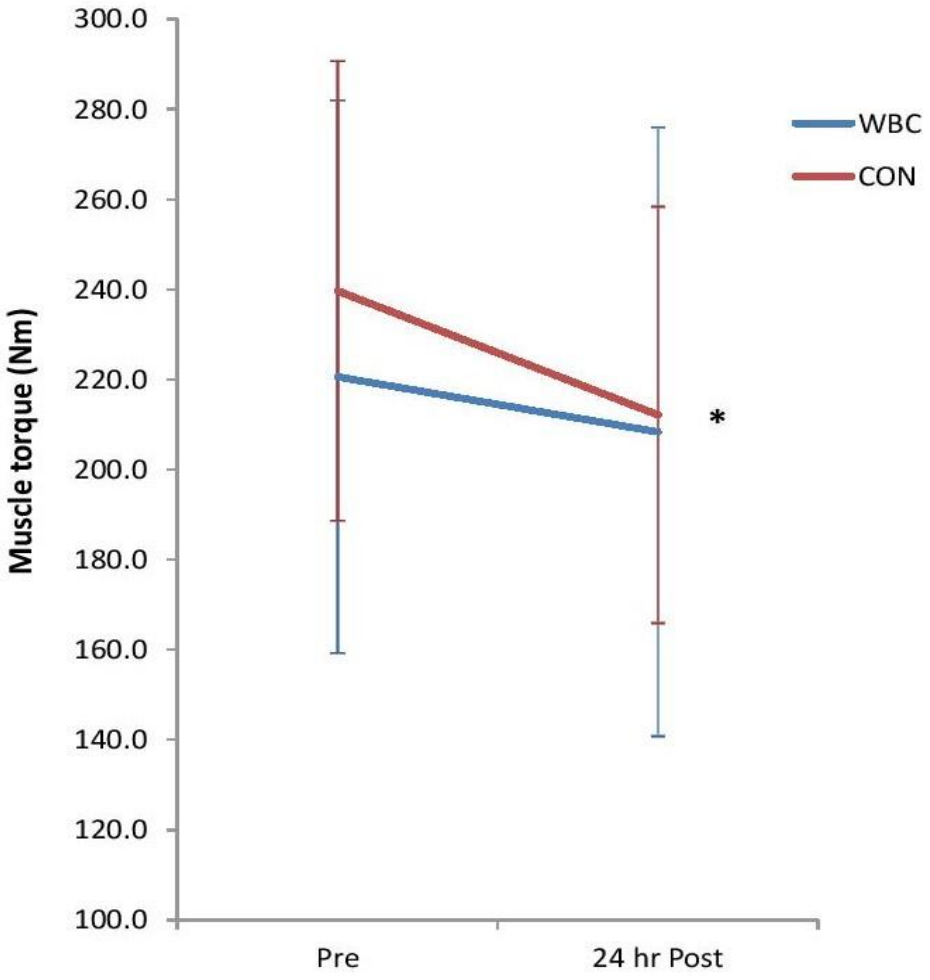


Figure 4.3.1: Maximal muscle torque response between WBC (n=26) and CON (n=15) groups. * P<0.05 for decrease in both groups. Data presented as means ± standard deviations.

Muscle Soreness

Soreness significantly increased from baseline to post-downhill run, 1 hour and 24 hour post-run for both WBC and CON groups, (overall effect of time $p<0.01$ for both groups) with a peak reached at 24 hours (47% for WBC; 44% for CON, Figure 4.3.2). There was no difference between groups over time (interaction effect, $p=0.87$).

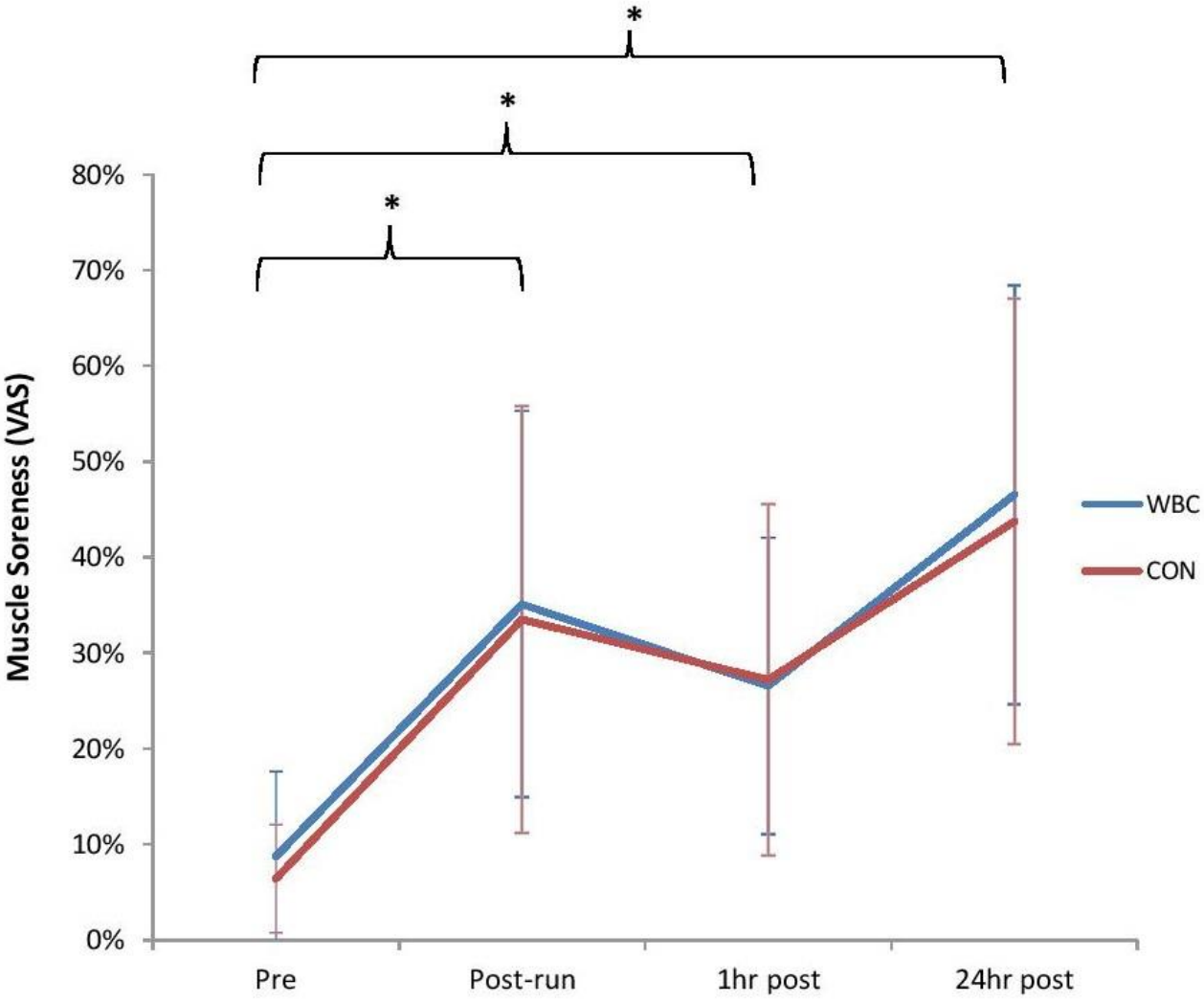


Figure 4.3.2: Muscle soreness response for WBC (n=26) and CON (n=15) groups. * P<0.01 for increases from baseline for both groups. Data presented as means ± standard deviations.

Creatine Kinase

Blood CK significantly increased from baseline to 24 hours post-run for both WBC (157.3 ± 110.4 IU/L vs. 418.4 ± 325.4 IU/L, p<0.01) and CON (176.3 ± 147.0 IU/L vs. 553.6 ± 286.1 IU/L, p=0.02, Figure 4.3.3). There was no overall difference between groups over time (interaction effect, p=0.78). The mean CK increases (baseline to 24 hour post) were 179.7% and 291.4% for WBC and CON participants respectively with no difference between groups (p=0.42).

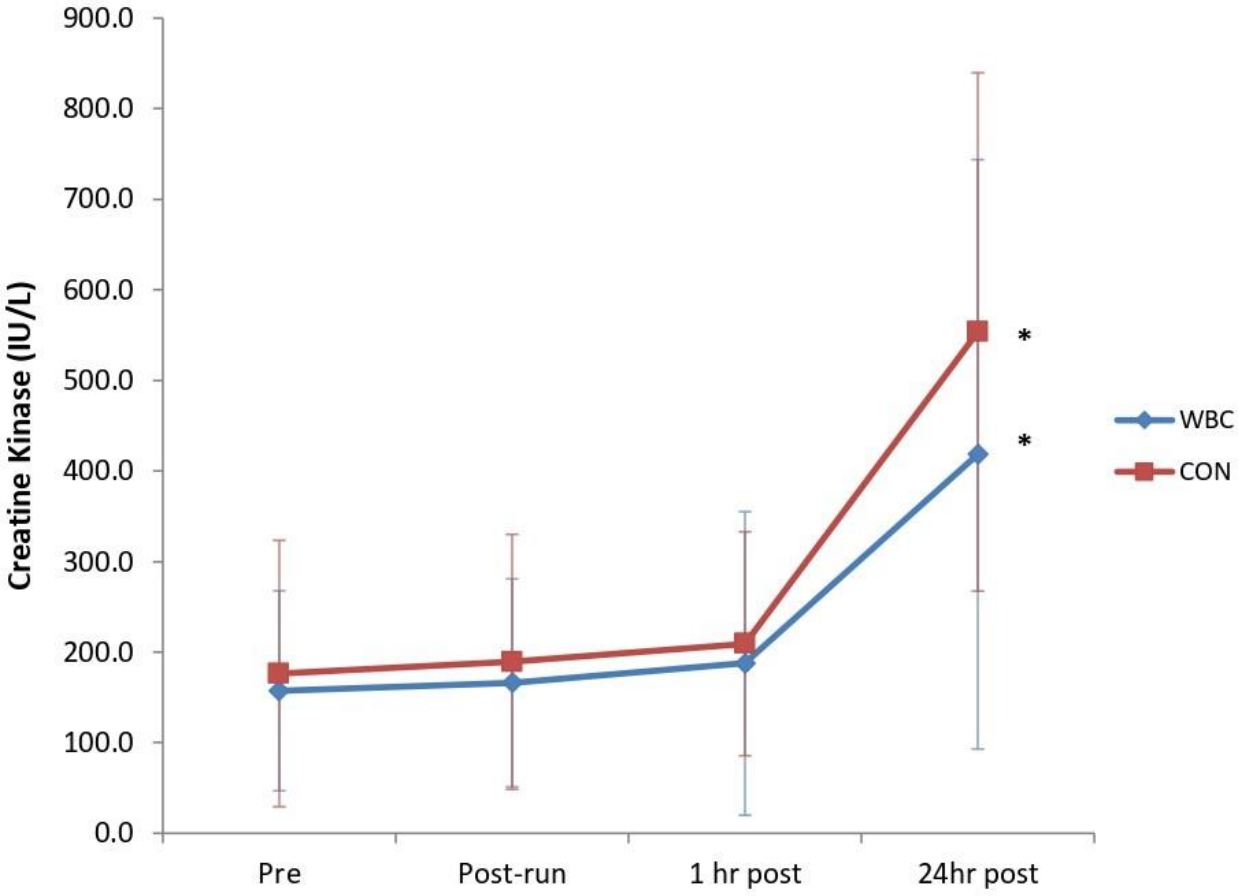


Figure 4.3.3: Blood CK response for WBC (n=26) and CON (n=15) groups. * P<0.05 for increase from baseline in both groups. Data presented as means ± standard deviations.

Tympanic Temperature

There was no difference in tympanic temperature from baseline to post-downhill run in the WBC group. For the WBC group, tympanic temperature significantly decreased post-WBC (36.8 ± 0.5°C vs. 36.4 ± 0.4°C; p<0.01). There were no differences for the CON group. There was a significant difference between WBC and CON groups at post-WBC (36.4 ± 0.4°C for WBC; 36.7 ± 0.3°C for CON, p=0.01, Figure 4.3.4).

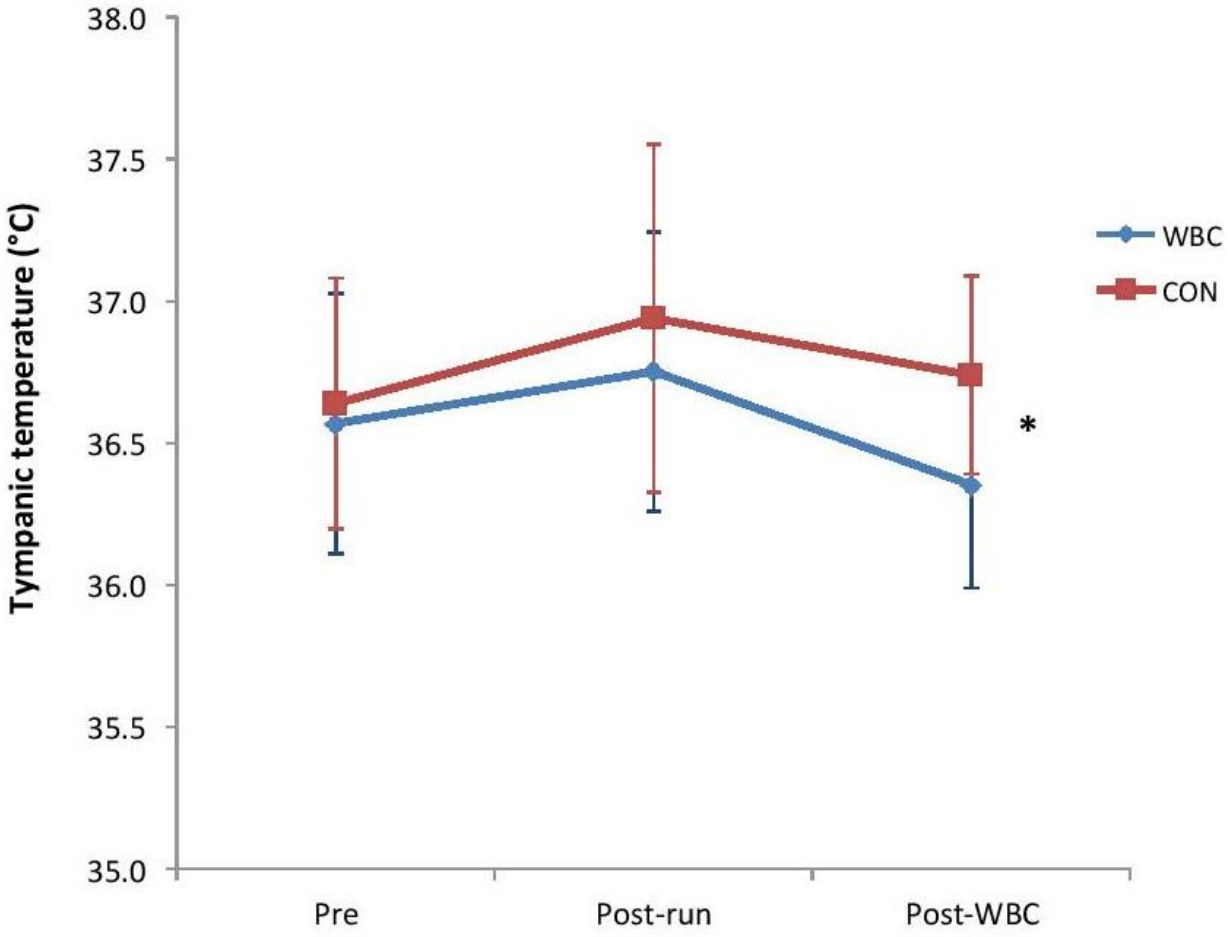


Figure 4.3.4: Tympanic temperature response for WBC (n=26) and CON (n=15) groups. * P<0.01 for difference between groups at post-WBC. Data presented as means ± standard deviations.

Skin Temperature

There was no difference in weighted mean skin temperature from pre to post-run in either group. Skin temperature significantly decreased 5 minutes post-cryotherapy for the WBC group (32.8 ± 0.9°C vs. 27.3 ± 1.5°C; p<0.01) whilst there was no difference for the CON group (Figure 4.3.5).

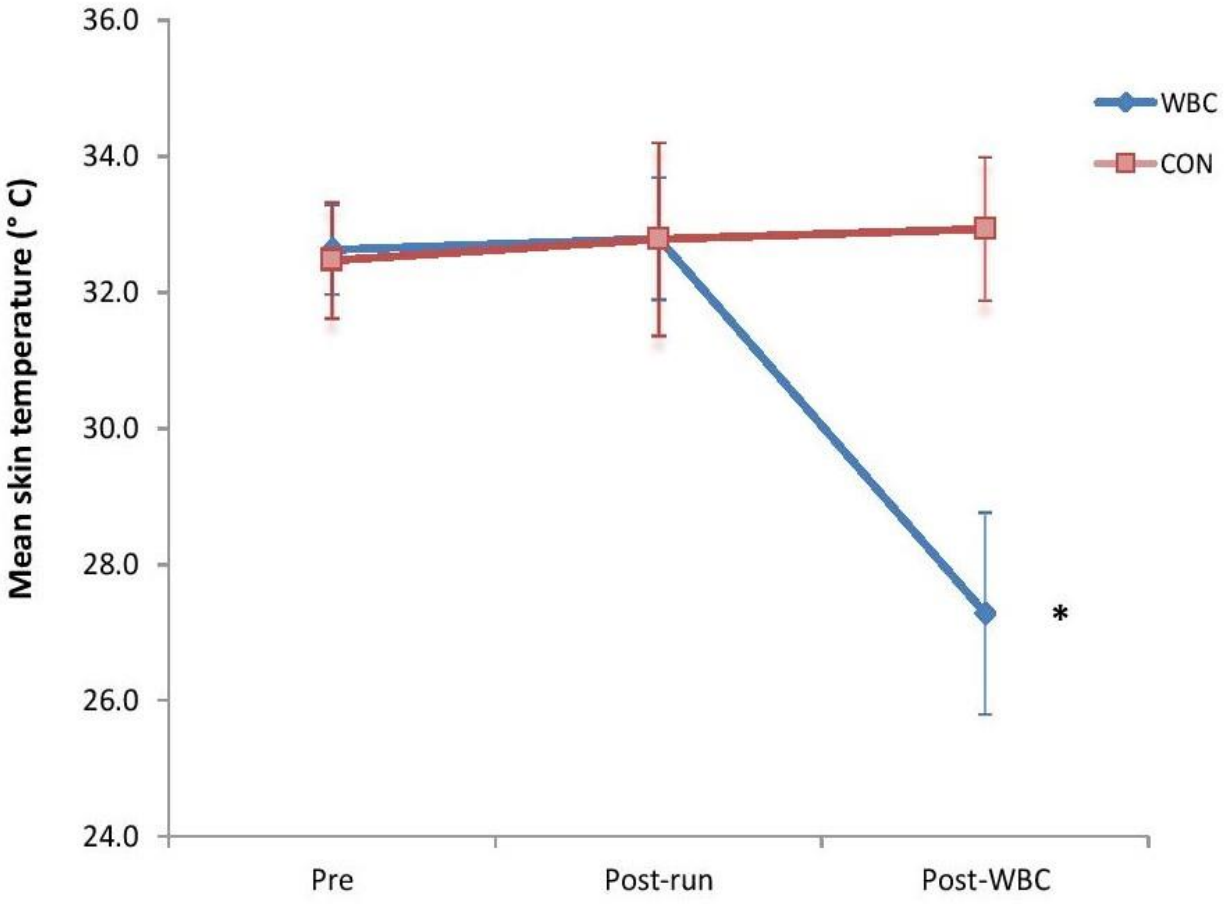


Figure 4.3.5: Skin temperature response for WBC (n=26) and CON (n=15) groups. * P<0.01 for decrease in WBC group. Data presented as means ± standard deviations.

Haemoglobin

There was no difference in haemoglobin for the WBC group (effect of time, p=0.23). There was no significant difference for the CON group, although there was a slight decrease at 24 hours post, approaching significance (p=0.06). There was no difference between groups over time (interaction effect, p=0.31). Haemoglobin results are presented in Table 4.3.1.

Table 4.3.1: Haemoglobin results for WBC (n=26) and CON (n=15) participants. Data presented as means ± standard deviations.

	Pre (g/L)	Post-run (g/L)	1hr post (g/L)	24hr post (g/L)
WBC	148.9 ± 16.7	149.4 ± 12.6	151.2 ± 11.5	146.6 ± 20.2
CON	147.0 ± 21.6	153.5 ± 18.4	152.1 ± 15.4	142.3 ± 11.7

VAS Wellbeing

Wellbeing scores did not significantly change between any paired time point for the WBC group, although the overall time effect approached significance (p=0.06). There was no difference for the CON group (p=0.44) and no interaction between group and time (p=0.53). VAS wellbeing scores are displayed in Table 4.3.2.

Table 4.3.2: Wellbeing scores for WBC (n=26) and CON (n=15) groups. Data presented as means ± standard deviations.

	Pre	Post-run	1hr post	24hr post
WBC	83.8% ± 16.7	82.3% ± 16.0	85.5% ± 15.0	81.1% ± 20.2
CON	78.9% ± 21.6	80.8% ± 18.4	81.2% ± 19.2	78.1% ± 16.9

Thermal Comfort

Thermal comfort significantly increased post-run for both groups ($p<0.01$) before decreasing post-WBC (1hr post run) to scores not different to baseline. There was no difference between groups over time (interaction effect, $p=0.86$), including at post-WBC ($p=0.29$). The mean thermal comfort scores are displayed in Table 4.3.3.

Table 4.3.3: Thermal comfort scores for WBC (n=26) and CON (n=15) groups. Data presented as means ± standard deviations.

	Pre	Post-run	Post-WBC
WBC	-0.5 ± 0.7	0.9 ± 0.7	-0.5 ± 0.8
CON	0.0 ± 0.9	1.4 ± 0.5	-0.1 ± 0.8

Heart Rate

Heart rate significantly increased post-downhill run in both groups ($p<0.01$) before returning to levels not different to baseline. There was no difference between groups over time (interaction effect, $p=0.28$). The heart rate response is displayed in Table 4.3.4.

Table 4.3.4: Heart rate (bpm) responses for WBC (n=26) and CON (n=15) groups. Data presented as means ± standard deviations.

	Pre	Post-run	Post-WBC
WBC	62.0 ± 11.1	133.5 ± 21.3	65.6 ± 13.5
CON	64.1 ± 9.4	139.6 ± 13.9	63.0 ± 9.9

Effects of Age and Body Fat

Muscle Torque

The pre-post difference in torque was significantly affected by age (interaction effect, p=0.02). There was a significant decrease in OLD participants (178.3 ± 37.5 Nm, 95% CI [155.1, 201.5] vs. 155.7 ± 49.2, 95% CI [125.2, 186.2], p=0.04, d=0.52) but no decrease in YNG participants (257.3 ± 60.7 Nm, 95% CI [224.3, 290.3] vs. 253.3 ± 54.0 Nm, 95% CI [223.9, 282.6], p=0.55, d=0.07), following WBC (Figure 4.3.6A). The pre-post difference in torque was not significantly affected by body fat (interaction effect, p=0.41). There was a trend for a slight decrease in torque for the HFAT group (187.9 ± 31.5 Nm, 95% CI [168.4, 207.4] vs. 167.6 ± 46.6 Nm, 95% CI [138.7, 196.5], p=0.07, d=0.52) and no decrease for LFAT (247.5 ± 68.0 Nm, 95% CI [200.4, 294.6] vs. 238.0 ± 70.8 Nm, 95% CI [189.0, 287.0], p=0.2, d=0.14, Figure 4.3.6B)

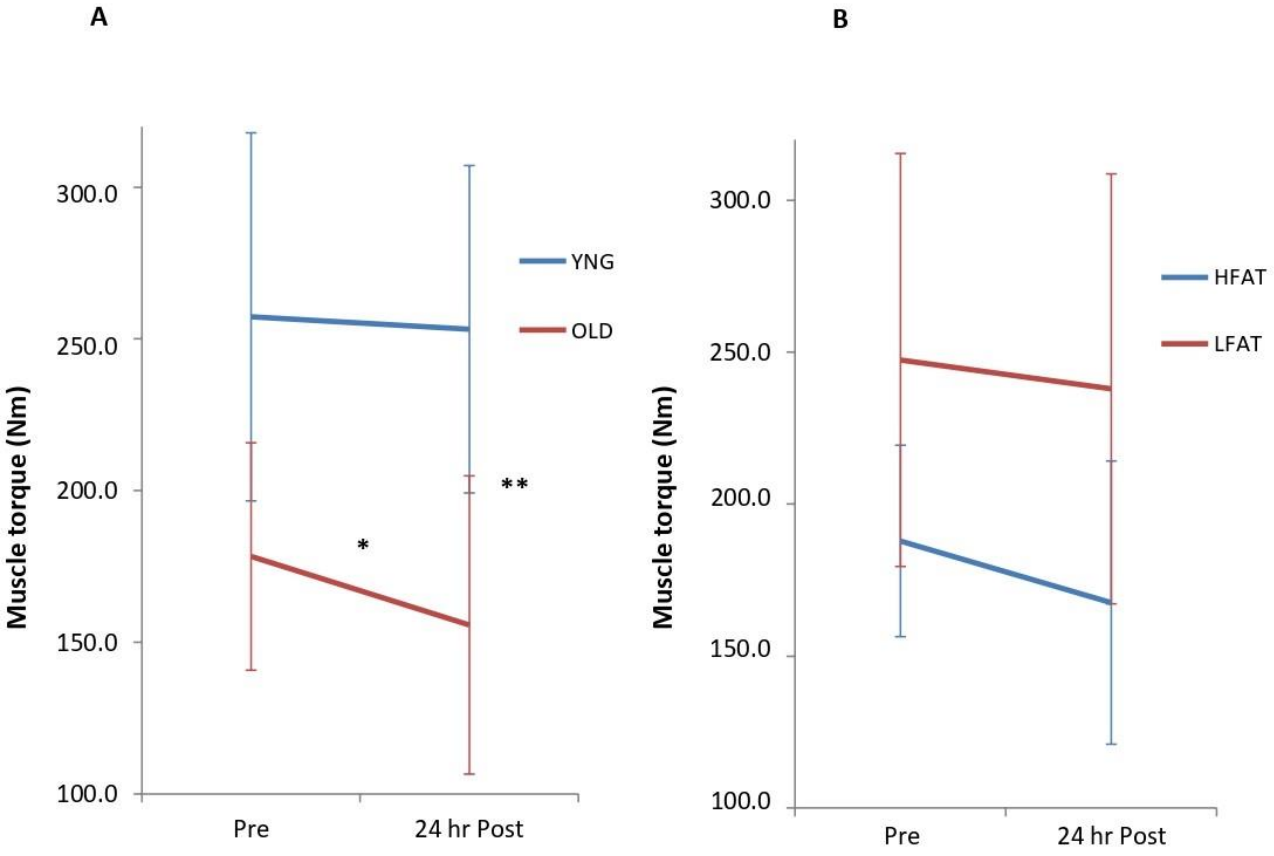


Figure 4.3.6: Maximal muscle torque response between YNG (<40 years, n=13) and OLD (≥45 years, n=10) participants (A), as well as between HFAT (≥20%, n=10) and LFAT (≤15%, n=8) participants (B) within WBC group. * P<0.05 for decrease in OLD; ** P<0.05 for interaction between age group and time. Data presented as means ± standard deviations.

Other Variables

The results for all other variables regarding age and body fat groups are summarised in Tables 4.3.5 and 4.3.6 respectively.

There was no overall effect of age ($p=0.68$) or body fat ($p=0.78$) on the muscle soreness response. The CK response was not affected by age group ($p=0.22$) and there was no significant difference between OLD and YNG at 24 hours post, when the highest CK value occurred ($p=0.13$). There was no effect of body fat on CK ($p=0.59$), including at 24 hours post ($p=0.16$). Tympanic temperature was significantly higher for YNG than OLD at post-WBC (36.5 ± 0.4°C vs. 36.1 ± 0.3°C $p<0.01$). There was no effect of body fat on tympanic temperature ($p=0.44$). There was no effect of age or body fat on weighted mean skin temperatures (p values>0.2). There was no effect of age group ($p=0.17$) or body fat ($p=0.22$) on wellbeing scores. For heart rate, there was a significant age group interaction ($p=0.00$) but no difference between groups post-WBC ($p=0.2$). The HR interaction effect for body fat group approached significance ($p=0.07$). There was no significant effect of age group on thermal comfort ($p=0.13$) while the effect of body fat group approached significance ($p=0.07$). There were no effects of age or body fat on haemoglobin (p values>0.8).

Table 4.3.5: Results for all variables other than muscle torque between OLD (≥45 years, n=10) and YNG (<40 years, n=13) WBC participants. * P<0.01 for difference in tympanic temperature post-WBC.

Variable	WBC Age Group	Pre	Post-run	1hr post (Post-WBC)	24hr post	P value for time x group interaction
Muscle Soreness	OLD	8.7% ± 7.2	28.6% ± 16.6	21.6% ± 15.8	42.6% ± 21.4	0.68
	YNG	10.1% ± 10.7	40.2% ± 22.7	31.3% ± 14.1	49.5% ± 21.8	
CK (IU/L)	OLD	149.5 ± 106.2	142.6 ± 94.5	175.9 ± 119.7	351.0 ± 283.3	0.22
	YNG	177.0 ± 111.9	196.1 ± 124.7	212.7 ± 194.2	502.1 ± 349.1	
Tympanic Temp	OLD	36.4°C ± 0.5	36.7°C ± 0.6	36.1°C ± 0.3 *	n/a	0.17
	YNG	36.8°C ± 0.4	36.8°C ± 0.5	36.5°C ± 0.4 *	n/a	
Skin Temp	OLD	32.6°C ± 0.6	32.2°C ± 0.7	27.4°C ± 1.7	n/a	0.21
	YNG	32.8°C ± 0.6	33.3°C ± 0.7	27.4°C ± 1.4	n/a	
VAS Wellbeing	OLD	88.5% ± 16.1	84.8% ± 13.3	87.7% ± 13.4	82.6% ± 21.6	0.17
	YNG	85.7% ± 10.5	84.8% ± 11.8	88.9% ± 8.2	85.1% ± 9.5	
Haemoglobin (g/L)	OLD	147.8 ± 15.7	144.8 ± 12.7	148.2 ± 15.0	143.6 ± 13.4	0.83
	YNG	150.2 ± 12.3	150.5 ± 10.1	154.5 ± 8.2	149.2 ± 11.9	
Thermal Comfort	OLD	-0.8 ± 0.8	0.8 ± 0.9	-0.3 ± 0.8	n/a	0.13
	YNG	-0.3 ± 0.6	1.0 ± 0.6	-0.6 ± 0.8	n/a	
Heart Rate	OLD	60.0 ± 11.0	122.3 ± 25.0	61.6 ± 13.5	n/a	0.00*
	YNG	63.5 ± 12.2	143.5 ± 13.9	69.3 ± 14.2	n/a	

Table 4.3.6: Results for all variables other than muscle torque between HFAT (≥20%, n=10) and LFAT (≤15%, n=8) WBC participants.

Variable	WBC Body Fat Group	Pre	Post-run	1hr post (Post-WBC)	24hr post	P value for time x group interaction
Muscle Soreness	HFAT	9.1% ± 6.8	31.9% ± 16.7	24.8% ± 17.0	44.0% ± 22.0	0.78
	LFAT	12.25% ± 12.4	41.9% ± 20.8	34.1% ± 14.3	55.8% ± 17.2	
CK (IU/L)	HFAT	153.7 ± 103.3	147.1 ± 93.2	181.1 ± 118.3	391.2 ± 254.4	0.59
	LFAT	205.6 ± 131.4	233.7 ± 141.9	249.1 ± 234.2	588.3 ± 413.4	
Tympanic Temp	HFAT	36.5°C ± 0.4	36.6°C ± 0.6	36.2°C ± 0.4	n/a	0.44
	LFAT	36.8°C ± 0.4	36.7°C ± 0.5	36.5°C ± 0.4	n/a	
Skin Temp	HFAT	32.7°C ± 0.7	32.4°C ± 1.0	27.1°C ± 2.0	n/a	0.60
	LFAT	32.9°C ± 0.6	33.1°C ± 0.7	27.3°C ± 0.9	n/a	
VAS Wellbeing	HFAT	82.2% ± 22.8	80.7% ± 21.6	81.7% ± 21.9	79.9% ± 25.9	0.22
	LFAT	89.5% ± 9.4	80.8% ± 14.3	89.3% ± 9.6	80.1% ± 20.9	
Haemoglobin (g/L)	HFAT	148.2 ± 13.0	148.7 ± 10.3	148.5 ± 10.9	147.5 ± 6.3	0.99
	LFAT	149.9 ± 17.5	149.4 ± 14.9	150.0 ± 15.7	149.4 ± 18.4	
Thermal Comfort	HFAT	-0.7 ± 0.8	0.7 ± 0.9	-0.2 ± 0.8	n/a	0.07
	LFAT	-0.4 ± 0.7	1.3 ± 0.7	-0.8 ± 0.9	n/a	
Heart Rate	HFAT	61.8 ± 8.9	126.3 ± 18.8	63.4 ± 9.3	n/a	0.07
	LFAT	65.6 ± 15.8	141.3 ± 23.4	66.0 ± 14.1	n/a	

4.4. Discussion

The main finding in this study was that whole body cryotherapy blunted the decrease in muscle torque following a downhill running bout that was observed in the control group, indicating that WBC may attenuate muscle damage and support post-exercise recovery. Young participants responded significantly better to WBC with regards to muscle torque retention when compared to the older participants. These results partially support the initial hypotheses, although there was little impact on the response to the downhill run and cryotherapy between participants of different body fat contents.

The 30 minute downhill run caused a significant decrease in muscle torque for both cryotherapy and control participants, which is consistent with previous downhill running studies (e.g. Malm *et al.,* 2004; Peake *et al.,* 2005). The average torque decrements were 6.4% and 11.5% for WBC and control respectively. The decrease for the cryotherapy group is less severe than typically seen in other downhill running studies and this moderation effect could be significant in a sports and performance context.

This is the first study to demonstrate a positive effect of a single treatment of WBC on muscle performance 24 hours post-EIMD. Previous studies that have observed beneficial effects of WBC for treating EIMD either used multiple treatments (Fonda & Sarabon, 2013; Hauswirth *et al.,* 2011) or partial body cryotherapy (Ferreira-Junior *et al.,* 2014), where the head is not exposed to extreme cold, therefore having different physiological mechanisms (Bouzigon *et al.,* 2016). Experiencing beneficial effects using just a single treatment of WBC highlights the potential effectiveness of the intervention and is likely to be more economical and feasible than applying multiple treatments. Caution should be exerted when interpreting the findings, since the overall interaction effect for group and time was non-significant ($p=0.10$). Nonetheless, with a reasonably strong effect size of 0.67 for difference between groups in torque reductions, it is likely that such alleviations of muscle strength decrement following eccentrically biased exercise would result in superior athletic recovery.

The other EIMD markers however do not indicate support for the application of WBC post-downhill run. There was no difference in muscle soreness between the

cryotherapy and control groups. The debate on whether WBC is effective in reducing muscle soreness has been highlighted previously (Bleakley *et al.,* 2014; Costello *et al.,* 2015) and by the discrepant findings between studies demonstrating benefits (e.g. Fonda & Sarabon, 2013; Wilson *et al.,* 2018) versus studies that have not (Costello *et al.,* 2012; Ferreira-Junior *et al.,* 2015). The variety of EIMD protocols utilised in WBC studies makes it difficult to draw definitive comparisons and conclusions regarding the impact of WBC on muscle soreness. Further experimental research is warranted to clarify the potential impact of WBC on alleviating levels of muscle soreness.

There was no effect of WBC on the blood CK response post-downhill run. CK is a commonly used EIMD marker due to its ease of detection in the circulation and the indication of disrupted muscle membranes when its levels are elevated (Clarkson, 2002). The effects of WBC on CK levels are equivocal. The few studies that have demonstrated blunted CK responses (e.g. Banfi *et al.,* 2009; Ziemann *et al.,* 2012) utilised multiple WBC treatments which might induce more attenuation of CK levels. This study only applied a single treatment post-exercise, which is likely to more accurately reflect a busy athlete's training schedule. The lack of impact of WBC on plasma CK post-downhill run indicates significant muscle fibre disruption. Associated characteristics include disrupted sarcomeres and Ca^{+} homeostasis, metabolic disturbances, Z-line streaming, presence of inflammatory markers and undermined excitation-contraction coupling (Peake *et al.,* 2017; Tee *et al.,* 2007), effects consistent with the moderate reduction in muscle strength that was still observed in the WBC group. It is therefore evident that many of the physiological effects of muscle damaging exercise were present in the WBC participants for this study. WBC was also ineffective in enhancing mental wellbeing scores despite other studies indicating otherwise (Pawik *et al.,* 2019; Szczepańska-Gieracha *et al.,* 2014).

The reductions observed in tympanic (0.4°C) and skin temperatures (5.5°C) post-WBC are comparable to previous studies (Costello *et al.,* 2012; Cuttell *et al.,* 2017; Kruger *et al.,* 2015) and consistent with the notion that WBC causes a pronounced vasoconstriction response, ensuring that blood flow is diverted away from the extremities to protect internal organs. It is not clear to what extent this thermoregulatory response can support recovery and performance post-exercise, especially since it is unlikely that the skin temperature decreased low enough to illicit a significant analgesic response. Assessing same day performance measures (e.g.

power tests) post-WBC may provide further understanding of how physiology responses post-cryotherapy might be linked to functional performance.

There was no impact of WBC treatment on either heart rate or haemoglobin, suggesting that WBC did not affect the oxygen carrying capacity of the blood post-downhill run. The parasympathetic response was unlikely to have been significantly different between the two groups since the heart rate for the WBC group post-cryotherapy returned to similar levels as that in the control group one hour post-downhill run (Table 4.3.4). As explained prior (section 2.2.5), an enhanced parasympathetic response and cardioprotective effect of WBC (Hausswirth *et al.,* 2013) could be an important benefit in supporting its use as a recovery tool. However, this was unlikely to have been a factor in this study.

Effects of Age and Body Fat

This is the first study to have investigated the effects of different ages and body fat contents on the response to WBC treatment for post-exercise recovery. Due to physiological differences between different age groups and body fat contents, it was hypothesised that younger and/or leaner men would respond more optimally to WBC post-exercise than older and/or higher fat individuals respectively. The main finding of interest was that the young WBC participants' muscle torques did not decrease 24 hours after the downhill run, whereas it decreased substantially for the older group.

Despite this significant finding, the young WBC participants still experienced EIMD, since significant muscle soreness and elevated CK were observed. Nonetheless, muscle torque is considered the most important marker of muscle damage (Warren *et al.,* 1999). The more favourable response to WBC post-exercise observed in the younger participants (<40 years) may have implications for coaching and training programmes with the potential use of WBC to support recovery following eccentrically biased muscle contractions. Half of the cryotherapy sample (13 of 26 participants) were aged below 40 and this sub-group did not experience muscle torque decrements to the extent of the participants aged 45 and above. Thus, WBC appeared to be particularly beneficial for the younger participants in a functional sense.

It is not clear why the younger participants would retain their muscle strength following the cryotherapy treatment more than the older participants. Owing to the

established effects of ageing, potential theories include enhanced blood vessel and flow response to the leg muscles, better motor unit/muscle fibre activation, less disruption of excitation-contraction coupling, higher muscle-tendon stiffness, higher testosterone, reduced inflammation and/or the placebo effect. Due to the common occurrence of sarcopenia in elderly individuals (McCormick & Vasilaki, 2018), it is conceivable that the discrepancy in muscle recovery potential between age groups post-WBC and exercise can be attributed to differences in muscle mass. Enhanced muscle cooling is unlikely owing to the unusual finding of lower tympanic temperatures in the older participants post-WBC. Such theories and aspects would be potential avenues for further research to help understand how age differences can impact response to WBC post-exercise.

The only other variable where there was a significant effect for age group was heart rate. This was expected since heart rate at rest and exercise naturally decreases with age (Knight & Nigam, 2008). However there was no difference between age groups post-cryotherapy (Table 4.3.5). Combined with the lack of difference in haemoglobin values, it is probable that ageing does not significantly impact the cardiovascular response to WBC post-exercise. Further physiological measures such as muscle blood flow or oxygenation may provide further insight into the potential influence of age on responses to cryotherapy.

Due to the insulatory effect of body fat, some difference in the response to cryotherapy treatments between high fat and low fat participants was expected in this study. The leaner participants maintained their muscle torque post-WBC more than those with higher body fat, indicating that body fat could detriment the damage and recovery response to WBC post-exercise. Caution should be exercised in concluding this, since the interaction effect of body fat group was non-significant. Whilst it has been suggested that higher body fat decreases heat loss during cold exposure (Young *et al.,* 1996), there were no differences between HFAT and LFAT in tympanic and skin temperatures, which contrasts findings from previous WBC studies (Cuttell *et al.,* 2017; Hammond *et al.,* 2014). A possible explanation for this discrepancy is that tympanic and skin temperatures were only assessed at one time point post-WBC in this study. There was also no significant influence of body fat content on any of the other variables, contradicting the initial hypothesis. Further studies looking at the influence of different body compositions on response to WBC following other

exercise bouts (e.g. sports fixtures, repeated sprints) might add more perspective on WBC applications for sports recovery.

Study Limitations

The sample included a mixture of athletes from different sporting backgrounds and individuals who exercise recreationally. Most participants had a relative VO_2 max of below 50 ml/min/kg, of which a substantial portion were aged below 40. It can therefore be assumed that a large proportion of the sample were not trained athletes. It was initially the intention to include a variety of fitness levels and body sizes, but this factor should be considered before applying the findings to higher level sports practice.

A possible limitation is that muscle damage markers (strength, soreness and CK) were not assessed beyond 24 hours post-exercise. Whilst this is the first study to examine the response to WBC following a downhill run, two previous studies have applied cold water immersions post-downhill run (Crystal *et al.,* 2013; Rossato *et al.,* 2015). Both observed peak muscle damage markers 24 hours post-run instead of 48 hours. Other downhill run studies have observed peak muscle soreness at 24 hours (Close *et al.,* 2005; Dolci *et al.,* 2015; Nelson *et al.,* 2004) and 48 hours (Baumann *et al.,* 2014; Chen *et al.,* 2007) without any meaningful difference between these first two days. The greatest inflammation and loss of muscle function also occurs within 24 hours (Owens *et al.,* 2018) and blood CK typically peaks at 24 hours (Close *et al.,* 2005; Miyama & Nosaka, 2004; Park & Lee, 2015). Additionally, the extent of muscle strength reduction indicates mild damage (only 11.5% decrease for control group) where torque typically recovers within 48 hours (Paulsen *et al.,* 2012). It is therefore conceivable that the damage response at 24 hours would be a reliable indicator of damage extent at 48 hours and any alleviation of EIMD at 24 hours would likely result in quicker recovery to baseline. Athletes who train/compete several days a week are usually more concerned about next day recovery to successfully engage in further training sessions.

Finally, the logistical challenge of transporting participants to the cryotherapy chamber could have impacted some variables (e.g. tympanic temperatures) so it was not possible to control all ambient conditions before and after WBC.

Conclusions

Overall, WBC may alleviate the muscle damaging effects following downhill running due to an attenuation of muscle torque decreases. Despite EIMD being present, younger participants could take advantage of using WBC to mitigate muscle torque losses following an eccentrically biased long duration exercise. Body fat does not appear to heavily influence responses to WBC post-downhill run; however leaner individuals may benefit more by retaining levels of muscle strength. Future research should focus on the mechanisms through which younger practitioners can benefit more from cryotherapy treatments following an EIMD bout and how this could support recovery and sport performance. Additionally, it would be useful to further explore the potential recovery benefits of single WBC treatments following other exercise protocols.

CHAPTER 5 – MAIN STUDY 2

The Comparative Effect of Different Timings of Whole Body Cryotherapy Treatment with Cold Water Immersion for Post-Downhill Run Recovery

5.1. Introduction

There is currently limited research which has identified with any clarity the optimum whole body cryotherapy (WBC) protocol for recovery post-exercise. Further, due to the morphological differences associated with different sports, as well as diversity in training backgrounds, it would be pertinent to individualise cryotherapy treatments. WBC is also an expensive and relatively unique treatment that is not easily accessible for the majority of the athletic population. It is therefore necessary to determine optimal cryotherapy treatments for maximal impact. Certain factors such as treatment duration, temperature, timing and frequency should be considered in the prescription of training and recovery programmes.

5.1.1. WBC Treatment Factors

It is unlikely that variations in WBC treatment temperature (typically ranging from -110°C to -140°C) or duration (typically 3 minutes) will have a significant influence on outcomes, as benefits from different temperatures have been reported in the literature. For instance, -110°C was used by Ferreira-Junior *et al*. (2015) and Kruger *et al* (2015), and -140°C was used by Fonda and Sarabon (2013). Additionally, limited studies have manipulated these two factors (section 2.6 contains a more in-depth discussion). It is thereby possible that the timing of cryotherapy treatment can have an influence.

As discussed in section 2.6.1, due to the limited research, it would be useful to determine at what stage of recovery post-exercise WBC still evokes beneficial effects. Studies examining the positive effect of WBC on muscle recovery have applied treatments from within 15 minutes post-exercise (Ferreira-Junior *et al.,* 2014; Ferreira-Junior *et al.,* 2015; Hausswirth *et al.,* 2011), up to 45 minutes (Fonda & Sarabon, 2013) and one hour post-exercise (Kruger *et al.,* 2015). Studies examining WBC later than one hour are rare and applying the treatment 24 hours post-exercise did not result in any beneficial effect on muscle torque or soreness (Costello *et al.,* 2012). It is conceivable that the 24-hour period after strenuous exercise represents a window when cryotherapy can intervene to influence muscle damage progression. Due to the nature of the acute inflammatory response, a longer delay in the treatment may be ineffective in mitigating muscle damage to benefit recovery. Thus, it may be

helpful to establish a specific time point beyond which cryotherapy should not be applied to induce meaningful recovery. Factors such as cool down, transport and treatment accessibility may present additional logistical challenges for athletes wishing to use cryotherapy. Research on the impact of delayed treatments beyond one hour is lacking and no study has yet to assess the influence of WBC treatment timing on the damage or recovery response to exercise.

5.1.2. Inflammatory response

WBC can have significant anti-inflammatory effects (Banfi *et al.,* 2009; Pournot *et al.,* 2011; Ziemann *et al.,* 2012), perhaps due to the stabilisation of muscle membranes (section 2.2.3). Whilst it remains unclear how such effects may improve function and performance, a reduced muscle breakdown appears feasible, therefore enabling the muscle contractile properties to retain their force generating capacities better following muscle damaging exercises (Ferreira-Junior *et al.,* 2014). Many of the studies that demonstrate reduced inflammation following WBC treatment tend to use multiple treatments (e.g. 10 sessions over 5 days; Ziemann *et al.,* 2012) which are likely to induce more potent anti-inflammatory effects than a single treatment. A single WBC treatment 45 minutes following high intensity running was recently reported to have no significant impact on inflammation (Krueger *et al.,* 2018). Therefore, the potential anti-inflammatory effect of singular treatments (which are more economical and realistic for typical training programmes) following a single exercise bout is subject to further evaluation.

The cytokine IL-6, a significant component of the body's inflammatory response, is a commonly used inflammatory marker in muscle damage studies and elevations have been demonstrated post-downhill running bouts (Dolci *et al.,* 2015; Fortes *et al*., 2013; Smith *et al.,* 2007). The literature suggests that there tends to be an association between levels of such inflammatory markers in the blood and common muscle damage markers such as creatine kinase (CK) (Bruunsgaard *et al.,* 1997; Fatouros & Jamurtas, 2016). Despite this correlation, the precise role of inflammatory markers such as IL-6 in the muscle damage response remains unclear. It is generally accepted that the release and presence of IL-6 in the blood is the consequence of multiple factors post-exercise, (e.g. release of leukocytes) and that such cytokines can function as inflammatory 'messengers' that induce further responses both within the muscle cells and systemically (Paulsen *et al.,* 2012; Peake *et al*., 2017). No study

has yet to discover any significant effect of single WBC treatments on CK levels, which has been corroborated by findings in this thesis (sections 3.5 and 4.3). Thus, it is possible that mechanisms other than muscle membrane damage are involved should a singular WBC treatment post-downhill run have an impact on IL-6 levels.

The literature has not demonstrated that singular WBC can attenuate levels of IL-6 post-EIMD. It would therefore be sensible to further explore the inflammatory effect (as assessed by IL-6) of WBC treatment following EIMD.

5.1.3. Limb Blood Flow

Cold exposure effects on limb blood flow post-exercise can have implications for muscle recovery due to reductions in oedema, metabolism and hypoxic cell death (Ihsan *et al.,* 2013; Mawhinney *et al.,* 2013). Despite abundant literature on the effects of cold water immersion (CWI) on limb blood flow, only one study to date has used the Doppler technique to assess limb blood flow post-WBC (Mawhinney *et al.,* 2017). This study compared the blood flow response between WBC and CWI, identifying that blood flow decreases were more prominent following water immersion than WBC. However, the study did not examine such responses in conjunction with muscle performance measures (e.g. strength).

The blood flow response post-WBC remains a relatively under-investigated area, therefore additional research in this field will provide more understanding into how WBC could support muscle recovery parameters following an eccentrically biased exercise model. The Doppler ultrasound technique shall thereby be a new variable introduced in this thesis as an avenue to assess the blood flow response post-cryotherapy.

5.1.4. Cryotherapy effects on Sleep

Another potentially important area of research within the field of cryotherapy is the possible impact on sleep. Studies have indicated that WBC can enhance sleep quality. Schaal *et al.* (2014) found that female swimmers' sleep duration was significantly higher during a fortnight of repetitive WBC treatment period than a control period without WBC. The authors suggested that perceived fatigue was somewhat lower during the WBC treatment period. Despite these positive findings,

there were no significant benefits to swimming performance and speed. Another French research group recently revealed that isolated WBC treatments following intensive running enhanced sleep quality in physically active men (Douzi *et al.,* 2018), as well as football players following training sessions, including fewer body movements (Douzi *et al.,* 2019).

Research investigating the effects of CWI on sleep is equivocal. Some studies demonstrate a beneficial effect on sleep parameters (Al Haddad *et al.,* 2012; Tabben *et al.,* 2018), whereas others indicate little or no benefit (Robey *et al.,* 2013; Skein *et al.,* 2018). Such studies have also examined the quality of sleep as an outcome measure following cryotherapy treatments, rather than as a potential variable affecting post-exercise recovery responses to cryotherapy.

Since the benefits of sleep on sports recovery have already been established, any favourable impact of whole body cryotherapy on sleep could be considerable in the context of sports performance. Additionally, the influence of differing timings of cryotherapy treatments on sleep quality remains under-investigated.

5.1.5. Comparisons with Cold Water Immersion

Another contentious area within the field of cryotherapy is the comparative effect of WBC against CWI, especially considering CWI is far more accessible and economical for athletes. Whilst WBC has the advantage of imposing more extreme temperature on its users, thereby creating a larger temperature gradient between the body surface and the surroundings, CWI creates a hydrostatic effect, which has been argued to augment the effect of alleviating muscle swelling (White & Wells, 2013). Furthermore, cold water has a higher thermal conductivity than cold air, which has a higher potential to extract more heat from the body (Bleakley *et al.,* 2014). Several effects of CWI have already been established post-muscle damaging exercise (further reviewed in section 2.3.2). Some studies have directly compared the effects of WBC with CWI on recovery parameters post-exercise, with contrasting findings (Abaidia *et al*., 2017; Hohenauer *et al*., 2017; Hohenauer *et al.,* 2020; Qu *et al.,* 2020; Wilson *et al*., 2018; Wilson *et al.,* 2019). Two studies to date have examined recovery post-downhill run using CWI (Crystal *et al.,* 2013; Rossato *et al.,* 2015), again with discrepant findings since the latter study indicated benefits from CWI, whereas the former did not.

Further research is warranted in this area to compare these two cryotherapy methods on the effects of damage and recovery markers post-exercise. As explained in section 4.1, downhill running remains an under-investigated exercise protocol in the context of cryotherapy. Therefore, this study will aim to build on findings from the previous downhill run study (chapter 4) by exploring the comparative effects of WBC and CWI on muscle damage post-downhill run, as well as leg blood flow, inflammation and sleep. Clarifying a preferred timing of treatment may also assist athletes and coaches in determining an optimum protocol for WBC, which could represent a significant step in the constant strive for performance advantages.

Consequently, the objectives of this study were as follows:

1. To examine the influence of different timings of whole body cryotherapy treatment on muscle damage markers, thermoregulatory responses, leg blood flow, inflammation and sleep post-downhill run.

2. To compare the effects of whole body cryotherapy with cold water immersion on muscle damage markers, thermoregulatory responses, leg blood flow, inflammation and sleep post-downhill run.

5.2. Methods

5.2.1. Participants

A sample size calculation revealed that 28 participants would be appropriate to detect an effect. Thirty-three male volunteers (mean ± SD age 37.0 ± 13.3 years, height 1.76 ± 0.07m, body mass 79.5 ± 13.7 kg) completed this study, which adopted an independent groups design. Nine participants were randomly assigned into WBC1, which underwent WBC treatments one hour post-downhill run. Eight were assigned into WBC4, which underwent treatments 4 hours post-downhill run. An additional 8 participants were assigned to cold water immersion (CWI) one hour post-run and 8 were assigned as control (CON). All participants were of a suitable fitness level, partaking in regular physical activity and did not present any of the contraindications (Appendix 2). Sample characteristics for each group are summarised in Table 5.2.1. Prior to assessment, all participants had their blood pressure assessed and completed and signed a health questionnaire and informed consent form (Appendices 3 and 4). Ethical approval was obtained from the University of Northampton Graduate School Research Ethics Committee.

Table 5.2.1: Summary of characteristics for whole body cryotherapy 1 hour (WBC1), 4 hour (WBC4), cold water immersion (CWI) and control (CON) participants. Data presented as mean ± SD.

	WBC1 (n=9)	**WBC4 (n=8)**	**CWI (n=8)**	**CON (n=8)**	**OVERALL (n=33)**
Age (yrs)	35.3 ± 14.9	27.8 ± 5.9	38.1 ± 12.5	47.2 ± 12.0	37.0 ± 13.3
Height (m)	1.76 ± 0.08	1.76 ± 0.08	1.78 ± 0.05	1.73 ± 0.04	1.76 ± 0.07
Body mass (kg)	89.5 ± 20.8	75.8 ± 8.3	77.6 ± 7.0	74.0 ± 8.2	79.5 ± 13.7
Body mass index (kg/m^2)	28.6 ± 4.9	24.6 ± 2.5	24.6 ± 2.1	24.7 ± 2.6	25.7 ± 3.6
Body fat %	23.2 ± 7.0	18.0 ± 3.2	21.1 ± 4.3	21.7 ± 3.9	21.1 ± 5.1
Absolute VO_2 max (l/min)	3.52 ± 0.4	3.66 ± 0.42	3.40 ± 0.6	3.35 ± 0.24	3.41 ± 0.44
Relative VO_2 max (ml/min/kg)	40.4 ± 6.1	43.2 ± 7.7	44.2 ± 5.1	45.3 ± 3.86	43.3 ± 5.4

5.2.2. Initial Trial

All participants were asked to refrain from alcohol and strenuous exercise for 24 and 48 hours respectively prior to all trials. For the first trial, participants' anthropometric characteristics were assessed. A skinfold assessment for body fat percentage estimation was conducted as per the previous study (section 4.2).

The same procedure of familiarisation to the isometric muscle torque assessment was followed as in the previous study (section 4.2). Participants then performed their progressive VO_2 max assessment to volitional exhaustion with the same procedure followed as the previous study (section 4.2). Following completion, participants were allowed fluids and a cool down period. The absolute and relative VO_2 max values were reported and 60% of the absolute VO_2 max was calculated.

Participants then completed a muscle torque assessment on the Biodex dynamometer, involving four maximal contractions as explained prior (section 3.2). The highest torque produced from the four contractions was determined as the individual's pre-torque score. Participants were finally provided a sleep watch (Fitbit Inspire, California) and a questionnaire (Appendix 9) with instructions for use and completion, before being allowed to leave the laboratory.

5.2.3. Main Trial – whole body cryotherapy, cold water immersion and control participants

Within two weeks of the first trial, participants returned to the laboratory (ambient temperature 20 ± 0.5°C) to perform their main trial. Participants arrived in a rested and hydrated state and were instructed to avoid caffeine for four hours prior to the session. Participants lay down on a massage bed in a supine position for subsequent assessment of their right femoral artery blood flow via Doppler ultrasound *(*as explained in section 3.2*).* Tympanic temperatures and thermal images (for subsequent skin temperature assessment) were taken as explained previously (sections 3.2 and 4.2). Whole blood samples were acquired by venepuncture into 6ml vacutainer tubes for the subsequent measurement of IL-6, as explained in section 3.2. Participants then provided a fingerstick blood sample for the measurement of their CK levels. Their muscle soreness, thermal comfort and mental wellbeing were subsequently assessed via visual analogue scales (VAS).

Participants performed their 30 minute downhill run at a 15% decline following the exact same procedure as described in the earlier study (section 4.2), with target heart rate predetermined from the VO_2 max vs. heart rate relationship so that a running intensity corresponding to 60% of their VO_2 max was maintained. After completion of the running bout, tympanic and skin temperatures were immediately assessed as described previously, followed by CK, muscle soreness, thermal comfort and mental wellbeing.

The previously assigned groupings of participants determined the procedures and timings that followed. The one hour whole body cryotherapy participants were transported to the Chris Moody Centre for their subsequent WBC treatment at -120°C scheduled for 60 minutes post-downhill run, as explained in section 4.2. The four hour participants were allowed to leave the laboratory and instructed to arrive at the Chris Moody Centre ahead of their scheduled WBC treatment four hours post-downhill run, whilst maintaining hydration status and avoiding caffeine and alcohol. The same whole body cryotherapy procedures were followed as explained in sections 3.3 and 4.2. Control participants remained seated in the laboratory under controlled conditions.

Cold water immersion participants underwent their treatments one hour post-downhill run in the same building that the run was performed. Participants were immersed for 10 minutes into a plastic 200 litre butt (Figure 5.2.1) filled with water up to the level of the iliac crest. The water temperature was maintained at 15 ± 0.5°C using the addition/removal of ice. This temperature was selected as it lies within the 10-15°C range that the majority of CWI studies have used revealing benefits post-exercise (Versey *et al.*, 2013). Durations of 10 minutes have also been frequently used in previous muscle damage and cold water immersion studies (Bailey *et al.,* 2007; Vieira *et al.,* 2016). Due to height differences, some participants were instructed to adjust their position in the water slightly to ensure immersion up to their iliac crest. Participants were also instructed to avoid excessive movements throughout the 10 minute immersion.

Thermal images were captured from each participant 5 minutes post-WBC/CWI treatment for the subsequent assessment of skin temperatures. Tympanic temperature, blood CK, and VAS scales for muscle soreness, thermal comfort and mental wellbeing were measured 10 minutes post-treatment as described previously. A whole blood sample via venepuncture was then obtained before femoral artery

blood flow was assessed by ultrasound. Control participants had the same variables assessed under controlled laboratory conditions at the same corresponding time points as the WBC 1 hour and CWI groups. Participants were then allowed to leave the laboratory.

Figure 5.2.1: Plastic water butt used for cold water immersion trials with a total capacity of 200L. Participants were immersed up to waist level in a water temperature of 15°C.

5.2.4. Final Trial

Participants returned to the laboratory 24 hours after the downhill run for their final assessments. A whole blood sample was obtained by venepuncture. Blood CK and VAS scales for muscle soreness and wellbeing were measured. Participants then underwent their post muscle torque assessment, following the same protocol as the previous trials, with the peak torque elicited from their right leg determined as the highest of four maximal contractions. The sleep watch and questionnaire were also returned for subsequent analysis of sleep data.

5.2.5. Measurement of Sleep

Sleep was assessed using motion and sleep tracking wrist watches (Fitbit Inspire, California) for four consecutive nights – three nights prior to the main downhill run trial and the night following. The main variable of interest was sleep efficiency which was calculated as follows:

Sleep efficiency (%) = (Total time in bed – time awake)/Total time in bed

Total time in bed was determined as the 'sleep duration' displayed on the watch data. The watch also provided the number of awakenings and time spent awake within each sleep duration. Fitbit devices use an accelerometer to capture body motion data which are converted to algorithms to identify patterns of motions regarding daily steps, energy expenditure and sleep. Participants were instructed to wear the watches from the time they went to bed and remove them once waking. A sleep questionnaire (Appendix 9) was also completed for the same nights that the Fitbit watch was worn. Participants assessed their overall sleep quality on a scale from 5-very good to 1-very poor.

The literature on the reliability of Fitbit watches to assess sleep is inconclusive, although some studies have indicated reasonable levels of reliability when compared to other means of measuring sleep, particularly Actiwatch accelerometers (Evenson *et al.,* 2015; Feehan *et al.,* 2018)

The study protocol is summarised in Figure 5.2.2.

5.2.6 Statistical Analysis.

All data was analysed using SPSS Version 26. Data for all variables was assessed for normal distribution by the Shapiro-Wilk test and extent of skewness and kurtosis. Data was transformed as appropriate (log or square root transformation) when data significantly deviated from normal distribution. Two way repeated measures ANOVAs were used to assess the interaction effect between treatment group (WBC1 vs. WBC4 vs. CWI vs. CON) and time for all major variables. Paired t tests and pairwise comparisons with a Bonferroni correction were applied within groups where necessary to examine differences between specific timepoints. Effect sizes (Cohen's d) were calculated for muscle torque, the main dependent variable of interest. Significance levels were set at 0.05.

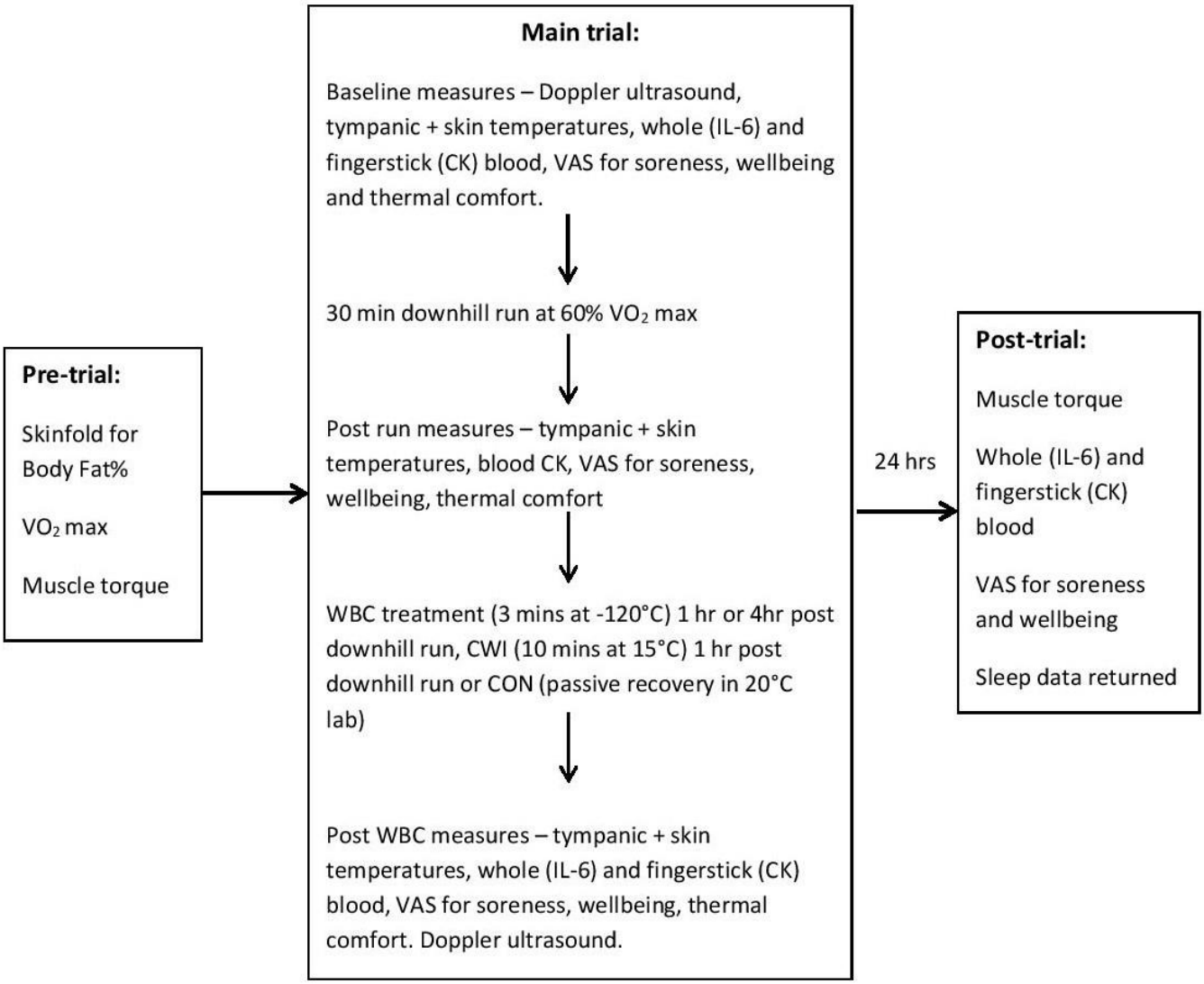


Figure 5.2.2: Protocol summary of measures for each trial in main study 2. Sleep was also assessed for 3 consecutive nights prior to main trial and night following. WBC – Whole Body Cryotherapy; CWI – Cold Water Immersion; CON – Control; CK – creatine kinase; VAS – visual analogue scale;

5.3. Results

Muscle Torque

There was a significant decrease in maximal isometric torque post-downhill run for the WBC4 and CON groups (WBC4, 263.5 ± 62.5 Nm vs. 231.0 ± 47.2 Nm, p=0.04, d=0.59; CON, 230.9 ± 53.9 Nm vs. 205.4 ± 52.6 Nm, p=0.00, d=0.48), a slight decrease for the WBC1 group that approached significance (258.2 ± 34.2 Nm vs. 243.8 ± 35.3 Nm, p=0.06, d=0.42) and no significant decrease for CWI (245.1 ± 76.8 Nm vs. 229.5 ± 72.7 Nm, p=0.15, d=0.21). The mean torque decreases were 14.4 ± 20.2 Nm (5.6%), 32.5 ± 37.0 Nm (10.9%), 15.6 ± 27.1 Nm (5.1%) and 25.5 ± 14.5 Nm (11.3%) for the WBC1, WBC4, CWI and CON groups respectively (Figure 5.3.1), with no differences between groups (p=0.45). The interaction effect for group and time was non-significant (p=0.45).

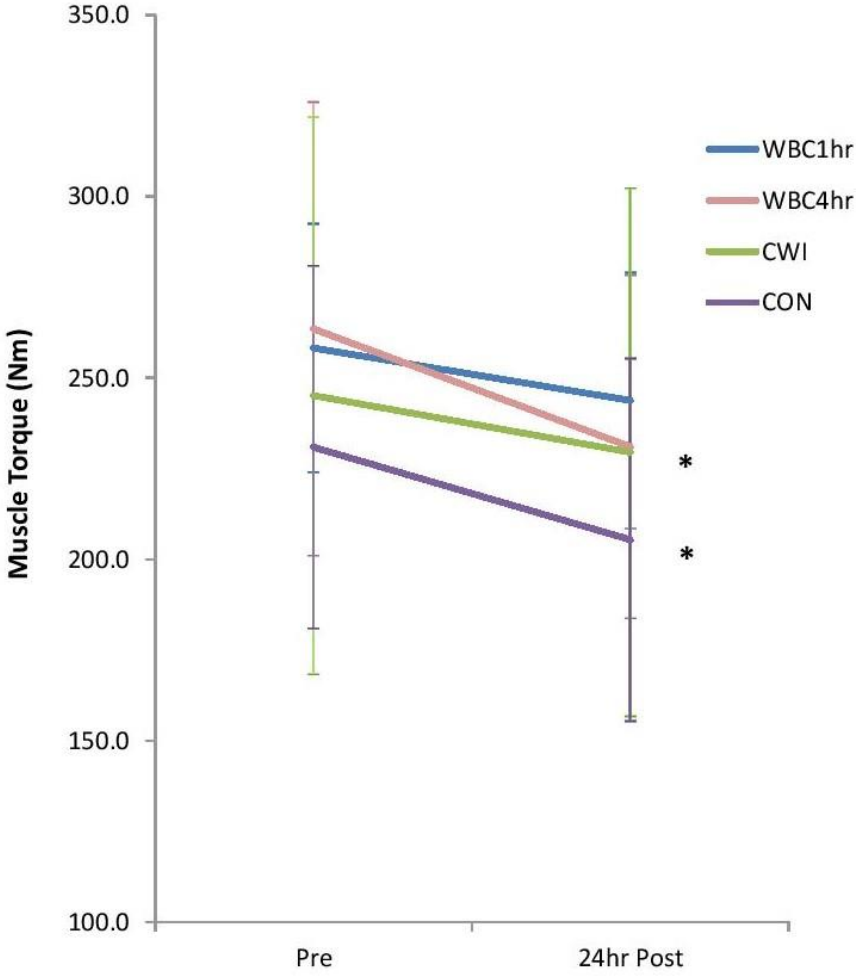


Figure 5.3.1: Maximal muscle torques for WBC1, WBC4, CWI and CON groups. *p<0.05 for decrease in WBC4 and CON only. Data presented as means ± standard deviations. N=33.

Muscle Soreness

Soreness significantly increased from baseline to post-downhill run and 24 hour post-run for all groups, (overall effect of time p=0.00 for all groups) with a peak reached at 24 hours for WBC1 (51%), WBC4 (49%) and CON (44%). The peak soreness for CWI was obtained post-downhill run (38%). There was no overall interaction between group and time (p=0.87). Pairwise comparisons revealed no differences between any of the groups at 24 hours post-run (all p values >0.6).

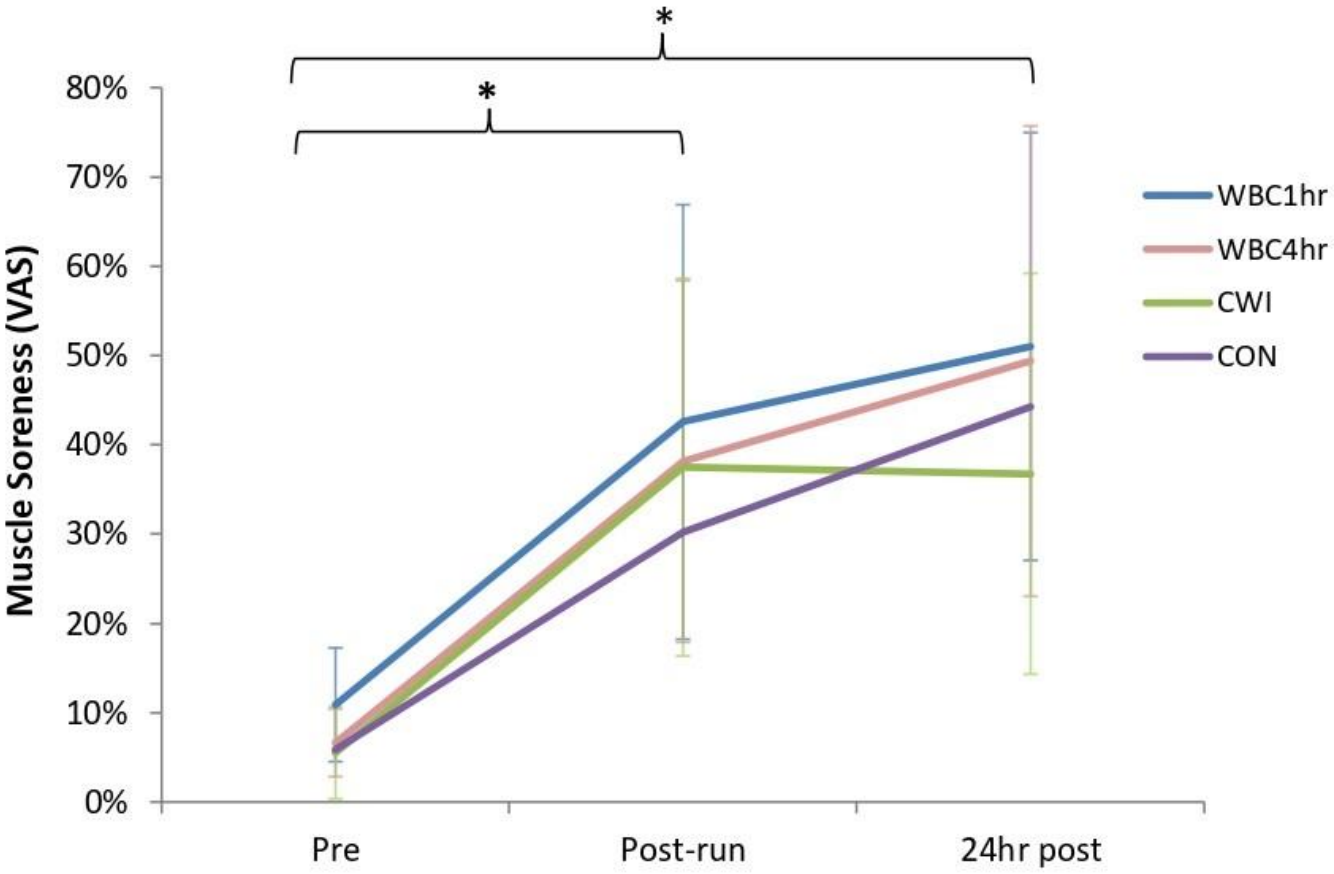


Figure 5.3.2: Muscle soreness response for WBC1, WBC4, CWI and CON groups. * P<0.05 for increase from baseline in all groups at post-run and 24hr post. Data presented as means ± standard deviations. N=33.

Creatine Kinase

Blood Creatine Kinase significantly increased from baseline at 24 hours post-run in all four groups (WBC1: 151.2 ± 88.2 IU/L vs. 442.8 ± 327.4 IU/L; WBC4: 163.1 ± 136.6 IU/L vs. 481.4 ± 170.5 IU/L; CWI: 182.5 ± 163.8 IU/L vs. 375.8 ± 229.8 IU/L; CON: 108.2 ± 39.9 IU/L vs. 465.3 ± 230.9 IU/L, p=0.00 for all groups, Figure 5.3.3). The peak CK value for the WBC4 group (564.3 ± 291.1 IU/L) was obtained post-WBC (4 hours post-run). There was no overall difference between groups over time (interaction effect, p=0.25). The mean CK increases (baseline to 24 hour post) were 188.8%, 401.5%, 194.5% and 314.6% for WBC1, WBC4, CWI and CON participants respectively with no difference between groups (p=0.35). There was no difference between groups at 24 hours post (p=0.76).

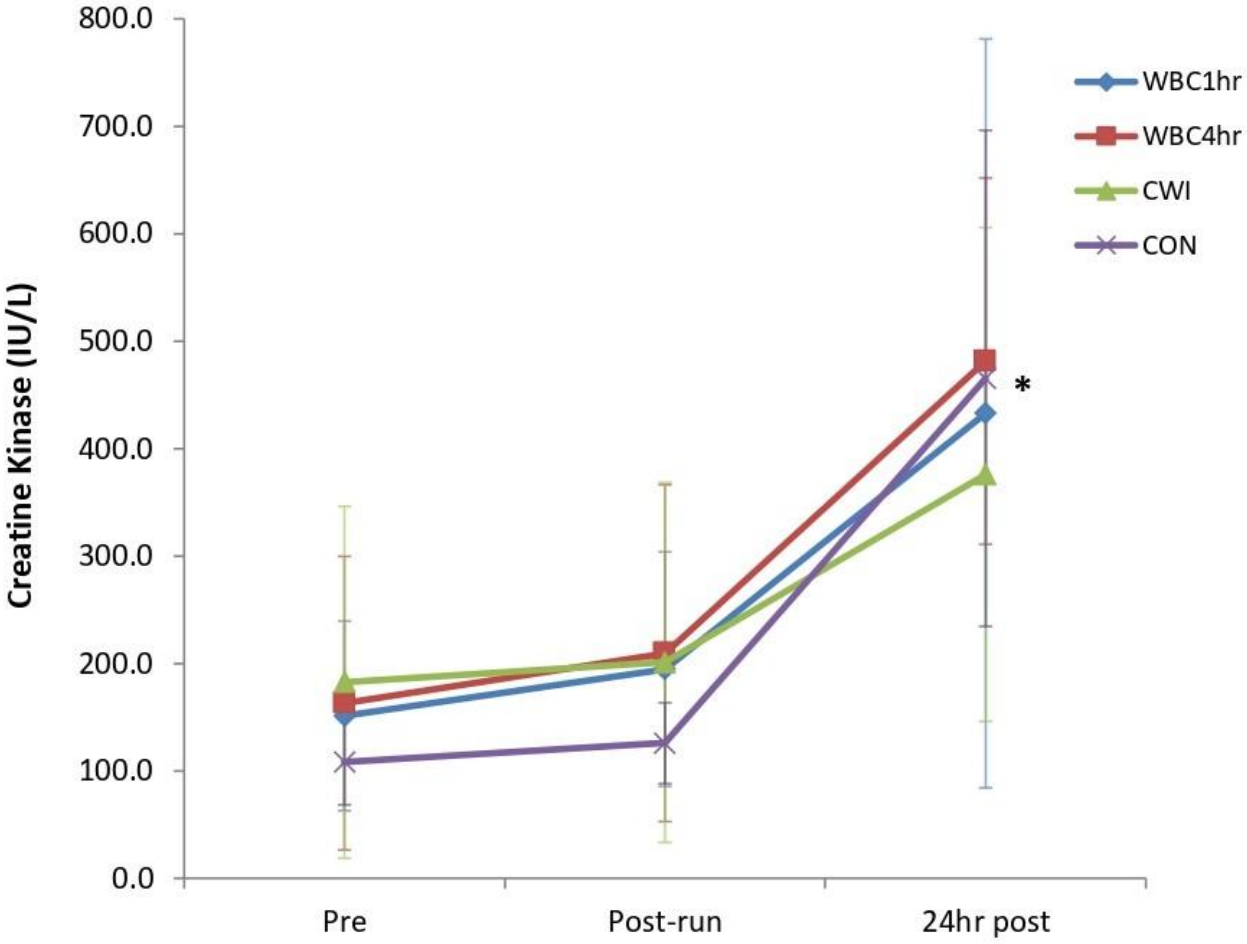


Figure 5.3.3: Blood CK response for WBC1, WBC4, CWI and CON groups. * P<0.05 for increase from baseline in all groups at 24hr post. Data presented as means ± standard deviations. N=33.

Tympanic Temperature

There was an overall significant effect of time for the WBC1 (p=0.05) and CON (p=0.03) groups but with no significant difference between paired time points. The decrease from post-run to post-WBC in the WBC1 group was non-significant (36.9 ± 0.8 °C vs. 36.3 ± 0.5°C; p=0.15). There was no difference in tympanic temperature within the WBC4 and CWI groups. The interaction between group and time was non-significant (p=0.17) and there were no differences between groups at post WBC/CWI (1 hour post-run for CON; p>0.1 for all pairwise comparisons).The tympanic temperatures for all groups are displayed in Table 5.3.1

Table 5.3.1: Tympanic temperatures for WBC1, WBC4, CWI and CON groups. Data presented as means ± standard deviations. N=33. * Measurement for WBC4 group taken post-WBC (approx. 4 hrs. post-run)

	Pre	Post-run	1hr post-run/ Post cryo*
WBC1	36.7 ± 0.3°C	36.9 ± 0.8°C	36.3 ± 0.5°C
WBC4	36.6 ± 0.3°C	36.8 ± 0.3°C	36.6 ± 0.3°C
CWI	36.7 ± 0.5°C	36.9 ± 0.3°C	36.8 ± 0.5°C
CON	36.6 ± 0.4°C	37.1 ± 0.5°C	36.7 ± 0.4°C

Skin Temperature

There was a significant decrease in skin temperature post-cryotherapy for WBC1, WBC4 and CWI groups (all p values <0.01) and a significant interaction between group and time (p=0.00) with no difference for CON (overall effect of time p=0.37, Table 5.3.2). The skin temperatures for the three cold treatment groups at 1hr post-run (post-cryotherapy) were significantly lower than CON (p=0.00). There was no difference between WBC1 and WBC4 post-cryotherapy. There was no significant difference between WBC1 and CWI (p=0.10) or between WBC4 and CWI (p=0.16) post-treatment.

Table 5.3.2: Weighted mean skin temperatures for WBC1, WBC4, CWI and CON groups. Data presented as means ± standard deviations. N=33. * Measurement for WBC4 group taken post-WBC (approx. 4 hrs. post-run)

	Pre	Post-run	1hr post-run/ Post cryo*
WBC1	32.2 ± 0.7°C	33.1 ± 1.2°C	27.3 ± 1.6°C
WBC4	32.3 ± 0.7°C	32.7 ± 0.8°C	27.4 ± 1.7°C
CWI	32.3 ± 1.1°C	31.8 ± 1.6°C	29.1 ± 1.1°C
CON	32.1 ± 0.9°C	32.5 ± 0.5°C	32.5 ± 1.3°C

Doppler Ultrasound

Femoral artery blood flows are displayed in Table 5.3.3. There was no difference in blood flow for WBC1, WBC4 or CON groups (all p values>0.5). Blood flow significantly decreased for the CWI group (195.0 ± 59.6 ml/min vs. 158.6 ± 80.3 ml/min, p=0.02). There was no significant interaction effect between group and time (p=0.19).

Table 5.3.3: Femoral artery blood flows for WBC1, WBC4, CWI and CON groups. Data presented as means ± standard deviations. N=33. * p<0.05 for decrease in CWI group.

	Pre (ml/min)	Post-run/cryo (ml/min)
WBC1	244.5 ± 50.5	248.8 ± 53.7
WBC4	235.4 ± 102.0	258.5 ± 161.0
CWI	195.0 ± 59.6	158.6 ± 80.3 *
CON	206.2 ± 53.9	214.3 ± 59.7

Plasma IL-6

IL-6 results are displayed in Table 5.3.4. The majority of samples were below the detectable limit of 0.49pg/ml. These samples were recorded as 0.25pg/ml for analysis purposes. There were no significant increases in IL-6 for any of the cold treatment groups (effect of time: WBC1, p=0.27; WBC4, p=0.15; CWI, p=0.42). There was a significant effect of time for CON (p=0.01) but no significant difference from pre to 1 hour post-run (p=0.13). There was no interaction between group and time (p=0.43).

Table 5.3.4: Plasma IL-6 response for WBC1, WBC4, CWI and CON groups. Data presented as means ± standard deviations. N=33. * Measurement for WBC4 group taken post-WBC (approx. 4 hrs. post-run). ** All samples 24hr post were below detectable limit so recorded as 0.25pg/ml for analysis.

	Pre (pg/ml)	1hr post-run/ Post cryo* (pg/ml)	24hrs post (pg/ml) **
WBC1	0.30 ± 0.13	0.47 ± 0.41	0.25 ± 0.00
WBC4	0.32 ± 0.18	0.59 ± 0.31	0.25 ± 0.00
CWI	0.25 ± 0.00	0.32 ± 0.15	0.25 ± 0.00
CON	0.25 ± 0.00	1.02 ± 0.88	0.25 ± 0.00

Sleep

Average durations in bed and sleep efficiency percentages each night are displayed in Table 5.3.5. There was no difference between groups over time for sleep efficiency (interaction effect, p=0.39). There was no effect of time for any group with no differences between paired time points (p>0.4 for all groups)

Sleep questionnaire ratings are displayed in Table 5.3.6. There was no difference between groups over time (interaction effect, p=0.62). There was no effect of time for any of the groups (p>0.2 for all groups).

Table 5.3.5: Sleep durations and % efficiency over 4 consecutive nights for WBC1, WBC4, CWI and CON groups. Downhill run and cryotherapy occurred between nights 3 and 4. Data presented as means ± standard deviations.

	Night 1 Duration (mins)	Night 1 Efficiency	Night 2 Duration (mins)	Night 2 Efficiency	Night 3 Duration (mins)	Night 3 Efficiency	Night 4 Duration (mins)	Night 4 Efficiency
WBC1	469.0 ± 74.0	95.0% ± 2.6	406.1 ± 79.2	93.7% ± 2.6	393.3 ± 105.7	93.7% ± 3.1	461.1 ± 52.6	94.5% ± 2.9
WBC4	380.0 ± 86.5	94.2% ± 3.3	421.4 ± 80.8	95.7% ± 3.5	362.4 ± 68.3	96.5% ± 1.8	405.8 ± 40.5	92.4% ± 8.1
CWI	484.8 ± 137.0	90.0% ± 14.5	481.2 ± 69.2	87.3% ± 19.4	495.4 ± 83.3	87.6% ± 16.8	530.2 ± 129.2	91.5% ± 10.4
CON	417.0 ± 90.1	97.0% ± 2.6	456.3 ± 44.6	97.2% ± 2.2	452.5 ± 35.0	97.0% ± 2.4	466.8 ± 54.6	97.0% ± 2.0

Table 5.3.6: Sleep questionnaire scores over 4 consecutive nights for WBC1, WBC4, CWI and CON groups. 5-'very good', 1-'very poor'. Downhill run and cryotherapy occurred between nights 3 and 4. Data presented as means ± standard deviations.

	Night 1	Night 2	Night 3 (night before main trial)	Night 4 (night after main trial)
WBC1	3.1 ± 0.9	3.2 ± 0.8	3.1 ± 0.9	3.4 ± 1.1
WBC4	2.7 ± 1.0	3.3 ± 1.5	3.2 ± 0.8	4.0 ± 0.6
CWI	3.1 ± 0.7	3.3 ± 0.5	3.4 ± 0.8	3.7 ± 0.5
CON	3.4 ± 1.0	3.7 ± 0.5	3.0 ± 0.6	3.6 ± 0.5

VAS Wellbeing

Wellbeing scores for each group are presented in Table 5.3.7. There was no overall interaction between group and time (p=0.55). There was no significant difference between any paired time points for any of the groups.

Table 5.3.7: VAS Wellbeing scores for WBC1, WBC4, CWI and CON groups. Data presented as means ± standard deviations. * Measurement for WBC4 group taken post-WBC (approx. 4 hrs. post-run)

	Pre	Post-run	1hr post run/ Post-cryo*	24hr post
WBC1	83.1% ± 9.3	84.7% ± 8.6	85.4% ± 7.8	85.0% ± 8.0
WBC4	77.5% ± 22.4	80.9% ± 15.0	85.0% ± 17.3	84.3% ± 15.2
CWI	79.6% ± 12.4	80.8% ± 10.1	80.9% ± 7.9	82.6% ± 9.7
CON	86.6% ± 17.4	88.8% ± 10.5	88.6% ± 12.5	86.8% ± 13.4

Thermal Comfort

Table 5.3.8 displays the mean thermal comfort scores for each group. Thermal comfort significantly increased post-run for WBC1, WBC4 and CWI groups ($p<0.05$) whilst the increase in CON approached significance ($p=0.07$). There was a significance decrease from post-run to post-cryotherapy/1hr post run for all groups ($p<0.05$). There was no difference between groups over time (interaction effect $p=0.35$) or post-treatment (all pairwise comparisons $p>0.15$).

Table 5.3.8: Thermal comfort scores for WBC1, WBC4, CWI and CON groups. Data presented as means ± standard deviations. * Measurement for WBC4 group taken post-WBC (approx. 4 hrs. post-run)

	Pre	Post-run	1hr post run/ Post-cryo*
WBC1	-0.9 ± 0.6	1.0 ± 0.5	-0.3 ± 0.5
WBC4	-0.6 ± 0.8	1.6 ± 0.8	-0.4 ± 0.8
CWI	-0.9 ± 0.6	1.1 ± 0.6	-0.9 ± 0.8
CON	0.0 ± 1.1	1.3 ± 0.7	-0.1 ± 0.6

5.4. Discussion

The principal aim of this study was to investigate the potential influence of WBC treatment timing on responses to muscle damaging exercise, since no prior study had manipulated WBC timing as a protocol factor. The main finding was that timing did not appear to have a highly significant impact on the outcomes following a downhill run, however there is evidence to suggest that taking cryotherapy treatments within an hour post-exercise is preferable for muscle strength recovery than delaying by several hours. Secondarily, WBC did not appear to be any more beneficial than CWI for all the assessed variables. There was also no impact of either cold treatment intervention on sleep.

Significant muscle damage occurred in the WBC4 and CON participants as observed by the significant decrease in muscle torque 24 hours post-run (approximately 11% decrease in these groups). The decrease of 5.6% for the WBC1 group is comparable to the WBC cohort in the previous downhill run study (chapter 4). Meanwhile, there was no significant decrease for the CWI group (5.1%, p=0.15), which would indicate that CWI can be a useful remedy to support post-exercise recovery, as supported by some earlier findings (Bailey *et al.,* 2007; Rowsell *et al.,* 2011). Since the slight decreases for WBC1 and CWI are very similar to the within subject day to day variance of 5.3% (pilot study 1, section 3.4), this would suggest that the WBC1 and CWI groups responded better with regards to muscle strength recovery. This supports the theory that delaying WBC treatment by several hours is disadvantageous due to the time course of the muscle breakdown mechanisms post-exercise, for instance infiltration of neutrophils, release of reactive oxygen species, as well as disruption of myofibrils, calcium handling and muscle contractile components (Peake *et al.,* 2017). However, the lack of difference between the WBC1 and CWI groups indicates that WBC is no more effective than CWI despite the more extreme cold temperatures. This supports earlier findings revealing no significant benefits of WBC treatments compared to CWI post-exercise (Hohenauer *et al.,* 2017; Hohenauer *et al.,* 2020*;* Wilson *et al.,* 2018).

Regarding muscle soreness, there did not appear to be an impact of WBC treatment timing since both WBC groups had near identical levels of muscle soreness 24 hours post-exercise. The lack of impact of WBC on muscle soreness post-downhill run corroborates the findings from the previous downhill run study (chapter 4), since

soreness post-WBC was no lower than CON for both studies. Although not significantly different from the other groups, CWI participants had the lowest peak of muscle soreness (38%, Figure 5.3.2) and were the only group where muscle soreness did not peak at 24 hours post. As explained previously (section 4.4), the peak muscle soreness typically occurs at either 24 or 48 hours. This was not the case in the current study, which opposes the findings from the two previous downhill run and CWI studies (Crystal *et al.,* 2013; Rossato *et al.,* 2015) where the peak soreness occurred at 24 hours post-run. It is conceivable that the soreness for the CWI group would have dropped further at 48 hours post-run, potentially favouring the use of CWI over WBC. Whilst the impact of WBC on muscle soreness remains contentious (Costello *et al.,* 2015), several findings have supported the use of CWI for alleviating soreness post-exercise (Ascensão *et al.,* 2011; Bailey *et al.,* 2007). This is possibly due to the fact that cold water imposes a hydrostatic effect which can further curtail swelling and efflux of metabolites (White & Wells, 2013).

There was no impact of either cryotherapy intervention on plasma CK levels. The overall CK values at 24 hours post were quite moderate (all groups <500 IU/L) and indicates the relatively mild extent of muscle damage caused by the downhill run. Whilst downhill running causes substantial muscle damage, the extent of damage is not as severe as other commonly adopted EIMD protocols such as drop jumps (Hohenauer *et al.,* 2020; Westerlund *et al.,* 2009) and isolated arm curls (Yoon & Kim, 2020). Neither cryotherapy intervention was successful in blunting the plasma CK response, regardless of treatment timing. It therefore appears that singular cryotherapy treatments (cold air or water) are insufficient to considerably alter blood CK levels post-exercise and affect the muscle membrane breakdown following exercise.

Summarising the three main muscle damage markers utilised in this body of work, it appears that WBC treatments one hour post-exercise may be preferable for muscle strength recovery than delaying the treatment by several hours. However, taking WBC one hour post-exercise is no more effective for treating EIMD than CWI. The findings from this study therefore highlights the contentious issue of determining which of the two cryotherapy modes is more effective for treating EIMD.

The thermoregulatory responses following cryotherapy are comparable to the previous downhill run study (chapter 4). For instance, skin temperatures post-WBC were almost identical to the previous study (27.3°C), regardless of treatment timing.

Due to the lack of difference between groups as well as post-treatment, it is unlikely that the tympanic temperature response would have any meaningful impact on the body's ability to recover from muscle damaging exercise. Whilst thermal images were taken 5 minutes post-treatment, it is unlikely that skin temperatures dropped substantially to induce an analgesic effect (Bleakley & Hopkins, 2010) in either of the cold treatment interventions. The skin temperature response is consistent with the well-established mechanisms of cold-induced vasoconstriction and consequent vasodilation (White & Wells, 2013), yet the implications of skin temperature reductions on subsequent performance parameters remain unclear. Furthermore, this study reveals that treatment timing does not influence the thermoregulatory response to WBC.

Although not statistically significant (p=0.10), the mean skin temperatures for WBC1 (27.3°C) were slightly lower than CWI (29.1°C) 5 minutes post-treatment. This is likely due to the fact that the CWI participants were only immersed in water up to the iliac crest, thus significant decreases in skin temperatures would have only been observed in the thigh and calf (data for specific body regions not shown). It remains to be seen whether varying the water immersion depth can impact recovery response post-exercise. Immersion up to waist level is a common protocol (Versey *et al.,* 2013) as well as being more tolerable for athletes and participants, yet full body immersion may allow for more reliable comparisons with WBC. Direct comparisons between WBC and CWI on thermoregulatory parameters have not commonly been investigated, although more pronounced skin temperature reductions have been reported following WBC compared to CWI (Costello *et al.,* 2012; Costello *et al.,* 2014). There are two major methodological differences between these studies and the present study. Firstly, the current study assessed mean whole body skin temperature whereas the previous studies measured just the thigh or calf. Secondly, Costello *et al.* utilised a more uncommon CWI protocol of 4 minutes duration with immersion up to the sternum.

As expected, there was a significant decrease in leg blood flow for the CWI group post-treatment, however there were no decreases for either WBC intervention. One proposed mechanism of WBC potentially aiding the alleviation of muscle damage is a reduced blood supply causing reduced muscle metabolism, thus reduced muscle breakdown (Ferreira-Junior *et al.,* 2014; White & Wells, 2013). Previous studies have demonstrated reduced muscle temperatures post-WBC (Costello *et al.,* 2012).

Although the impact of WBC on muscle metabolism per se remains inconclusive, reductions in muscle oxygenation post-cryotherapy have recently been revealed (Hohenauer *et al.,* 2020). In the current study there was no impact of WBC on femoral artery blood flow, regardless of the timing. One possible explanation for this unexpected finding is that the Doppler ultrasound measures in the WBC participants could have been affected by the logistics, since it was not possible to control all ambient conditions once the participants left the laboratory following their downhill runs. Since the cold water immersions occurred in the same building as the downhill run without any transporting being involved, the blood flow measures for these participants were likely to have been more reliable. One study has previously reported significant reductions in leg blood flow post-CWI and post-WBC but with more pronounced reductions following CWI (Mawhinney *et al.,* 2017). Besides the lack of exercise performance measures, one notable difference is that this prior study assessed femoral blood flow at more time points pre and post-treatment. Due to the need to transport WBC participants in the current study, it was not feasible to measure leg blood flow between the downhill run and the cryotherapy treatment without causing further treatment delay. Nonetheless, the finding that leg blood flow significantly reduced following CWI post-run might explain why CWI is potentially favourable for alleviating muscle soreness and/or swelling. A decreased blood flow to muscles post-exercise can help to reduce muscle swelling due to lower metabolic demands (Ihsan *et al.,* 2016). Muscle metabolism or swelling was not assessed in the current study, but the possibility of addressing these markers in further comparative studies between WBC and CWI remains a possible intriguing avenue for further research.

The IL-6 results would indicate that the downhill run did not cause a substantial inflammatory response, with an average peak of only 1.02 pg/ml for CON. This is in contrast with previous downhill run studies (e.g. Dolci *et al.,* 2015; Smith *et al.,* 2007) observing IL-6 values ranging from 3-12 pg/ml post-run; although lack of increases in IL-6 expression post-downhill running has also been observed (Malm *et al.,* 2004). The majority of the plasma samples had IL-6 levels below the detectable limit of the Bio-Plex immunoassay, which is an unexpected finding. One potential cause of a lack of substantial inflammatory response could have been the mild extent of muscle damage (only 11% decrease in torque for CON, plasma CK <500 I/L). Additionally, downhill runs of longer durations than 30 minutes may induce more pronounced increases in inflammatory markers, as was evident in the aforementioned downhill

run studies. Further research to clarify the potential impact of single cryotherapy treatments on inflammatory responses post-muscle damaging exercise would be useful.

Due to the established importance of sleep quality for athletic recovery and performance (Walsh *et al.,* 2020), it would be pertinent to further explore the potential impact of post-exercise recovery interventions on sleep. There was no impact of either cryotherapy intervention on sleep quality, as assessed by sleep watches and questionnaires. This contrasts previous WBC (Douzi *et al.,* 2018) and CWI (Tabben *et al.,* 2018) research. Purported claims for the potential benefits of cold applications on recovery and sleep post-exercise are core temperature reductions and parasympathetic re-activation (Douzi *et al.,* 2018), however these mechanisms were unlikely to have had a significant influence in the current study. Whilst treatment timings post-exercise were manipulated, it remains to be seen whether the effectiveness of cryotherapy interventions could vary according to the time of day. Due to athletes' busy schedules, some sessions may need to occur later during the day. It has been suggested that exercise and cryotherapy sessions later in the evening can have a more pronounced impact on subsequent sleep quality via modulation of sympathetic activity and hormonal circadian rhythms, thereby reducing motor activity and inducing more deep sleep (Douzi *et al.,* 2018). The cryotherapy treatments in the current cohort of participants occurred between 11am and 5pm, but it was not possible to undergo evening treatments due to the cryogenic chamber availability. Further research should therefore focus on the potential influence of manipulating exercise sessions and cryotherapy treatments according to the time of day to provide further insight on how cold interventions could mediate recovery improvements via sleep function.

Study Limitations

As highlighted earlier, due to the logistical challenge of transporting WBC participants for their treatments after the run and not being able to control all ambient conditions, it is probable that the Doppler ultrasound measures were not as reliable as anticipated. This might have particularly been the case for the WBC4 participants, since they were allowed to leave the laboratory for several hours before their cryotherapy treatments. This might explain the high standard deviations and the potentially unreliable results for this group in particular.

The reliability of the sleep measurements is also questionable, which could explain the lack of significant findings in this variable. Whilst previous studies have assessed sleep parameters post-WBC (Douzi *et al.,* 2018; Schaal *et al.,* 2014), this is the first cryotherapy study to use Fitbit watches. Wrist actigraphs have become a more popular mode in the assessment of sleep (Douzi *et al.,* 2019; Schaal *et al* 2014,). Polysomnography has also been utilised to good effect (Robey *et al.,* 2013). Due to the multi-objective nature of the study, such invasive assessment of participants' sleep would have arguably been impractical and ecologically invalid.

Additionally, only one marker of inflammation was assessed, with insignificant findings. Whilst the relevance of IL-6 for muscle damage and recovery is well established (Bruunsgaard *et al.,* 1997; Dolci *et al.,* 2015), other inflammatory markers implicated in EIMD include IL-1β, IL-10, CRP and TNF-α (Fatouros & Jamurtas, 2016; Krueger *et al.,* 2018; Pournot *et al.,* 2011). Measuring other markers would provide more insight into the specific local and systemic inflammatory responses, their time course and potential interactions between different inflammatory molecules following muscle damaging exercise.

Conclusions

In conclusion therefore, WBC treatments one hour post-exercise may be preferable for muscle strength recovery than taking the treatment four hours after exercise. This may imply a positive outcome for busy athletes with demanding schedules following exercise sessions, but could represent additional challenges to practitioners who do not have quick access to treatments. WBC treatment timing does not have a significant influence on post-exercise recovery parameters otherwise. Since WBC does not appear to be any more beneficial than CWI for post-downhill run recovery, CWI might be a preferred option due to its lower expense and relative ease of access. Additionally, there was no impact of either cold intervention on inflammation or sleep post-exercise. It would be beneficial for future research to focus more on comparing these two cryotherapy modalities following muscle damaging exercise bouts, particularly with regards to muscle swelling, blood flow and inflammatory markers.

CHAPTER 6 – MAIN STUDY 3

The Effect of Repetitive Whole Body Cryotherapy Treatment on Adaptations to a Strength and Endurance Training Programme

6.1. Introduction

Whole Body Cryotherapy (WBC) is a potentially useful, albeit expensive tool for post-exercise recovery, demonstrating a variety of effects such as reductions in pain, swelling and inflammation (Lombardi *et al.*, 2017). Whilst the treatment may benefit short term recovery (Hausswirth *et al.,* 2011), the majority of the sporting community are perhaps more concerned with strategies to enhance longer term responses throughout a training cycle. One area of controversy is whether WBC might hinder adaptive responses to training, a dilemma introduced previously in section 2.5.

Despite the demonstrated anti-inflammatory potential of WBC treatments (Ferreira-Junior *et al*., 2014; Ziemann *et al.,* 2012) the long term consequences of mitigating inflammation could be detrimental due to continual dampening of the adaptive responses. It is acknowledged that inflammation post-exercise is a means through which muscles can repair and regenerate (Fatouros & Jamurtas, 2016, Peake *et al.,* 2017), thereby facilitating training adaptations.

Previous cold water immersion (CWI) studies indicate that repetitive cryotherapy can blunt training adaptations, particularly with regards to muscle strength and hypertrophy (Fuchs *et al.,* 2020; Roberts *et al.,* 2015; Yamane *et al.,* 2006). Associated mechanisms include blunted arterial diameter gains and expression of anabolic signals (Yamane *et al.,* 2016), attenuation of myofibrillar protein synthesis (Fuchs *et al.,* 2020) and blunted increases in testosterone (Earp *et al.,* 2019). In contrast, other studies have not found such effects on endurance adaptations (Broatch *et al.,* 2017; Halson *et al.,* 2014). CWI has actually been demonstrated to augment endurance adaptations due to increased expression of PGC-1α, an established molecular marker in the activation of mitochondrial biogenesis (Ihsan *et al.,* 2014).

Four studies thus far have examined the potential impact of regular WBC treatment on adaptations to training. Assessing endurance markers such as VO_2 max and 20km cycling time trial performance, Broatch *et al.* (2019) discovered that three weekly WBC treatments did not influence adaptations to a four week cycling programme involving high intensity interval sessions. Growth factor benefits have been noted in volleyball and judo athletes following a two week period incorporating repeated WBC (10 total) and sports specific exercises (Jaworska *et al.*, 2018;

Jaworska *et al.*, 2021). The same research group recently revealed that repetitive WBC (three times a week for four weeks) could support strength gains via reduced myostatin levels, an established negative mediator of muscle adaptations (Jaworska *et al.,* 2020). These studies would therefore indicate no negative consequences of repetitive WBC application on adaptive responses, in contrary to some of the CWI studies.

Another factor to consider is the nature and type of exercises involved in the training programme. Based on the findings of the aforementioned studies, it is plausible that repetitive cryotherapy treatments hinder resistance training adaptations more than endurance adaptations. Physiological characteristics of resistance training adaptations include increased motor unit recruitment, myofibril cross sectional area and increased number of sarcomeres (Del Vecchio *et al.,* 2019; Shoenfeld, 2010). Whereas endurance training is typically associated with enhanced responsiveness of blood vessels and muscle capillary density, increased nitric oxide levels, arteriogenesis and mitochondrial biogenesis (Green *et al.,* 2012; Hawley *et al.,* 2018). Since it is established that the molecular pathways for each type of adaptation are distinct (Coffey & Hawley, 2017), impeding one type of pathway due to repetitive cooling does not necessarily imply a negative impact on the other pathway.

Additionally, the majority of the CWI and WBC studies highlighted previously only investigated training of a single type – i.e. interval or resistance training. Since there are different forms of training, it would be worth considering the relative impacts of cryotherapy in conjunction with various training methods. Programmes incorporating a combination of training methods are arguably more relevant for overall sports practice. For instance, football and other team sports typically focus on a variety of fitness attributes to ensure peak performance throughout the season, including speed, power, strength, agility and endurance (Stolen *et al.,* 2005; Wong *et al.,* 2010). Research examining the potential impact of repetitive WBC treatment in conjunction with concurrent training programmes remains scarce. Whilst the WBC study by Jaworska *et al.* (2018) utilised a combination of training methods, a two week training period is typically not long enough to induce sufficient adaptations (Kraemer *et al.,* 1998). As such, it does not reflect a characteristic programme in populations aiming to significantly enhance strength, endurance and/or other key fitness attributes. Furthermore, mesocycles targeting specific fitness/performance markers in sport usually last at least 5 weeks (Marrier *et al.,* 2018).

Certainly any negative impact of repetitive WBC on adaptive responses to training could outweigh the potential short term benefits, becoming counter-productive in the long run. This potential dilemma should be taken into consideration within the sports science community.

Due to the limited work and contrasting findings in this area, the overall impact of repetitive WBC treatment on chronic adaptations to training remains unclear. Thus, this study will aim to investigate the potential impact of repetitive WBC treatments on adaptations to a concurrent progressive 6 week training programme involving endurance, strength and power training. Elucidating this effect should enable scientists and sports practitioners to further evaluate the overall merit of WBC treatment for performance gains, as well as having implications for cryotherapy usage in relation to the periodization of training schedules.

6.2. Methods

6.2.1. Participants

Twenty male volunteers were initially recruited for the study, with 10 randomly assigned as cryotherapy and 10 assigned as control. Three of the cryotherapy participants withdrew due to injury during their programme. One of the control participants withdrew due to testing positive for COVID-19 and developing prolonged symptoms. Thus, 16 participants completed the study (mean ± SD age 33.4 ± 9.8 years, height 1.79 ± 0.05m, body mass 82.3 ± 9.8 kg). All participants were of a suitable fitness level for the purposes of the study and did not present any of the contraindications (Appendix 2). Sample characteristics for each group are summarised in Table 6.2.1. Prior to assessment, all participants had their blood pressure assessed and completed and signed a health questionnaire and informed consent form (Appendices 3 and 4). Ethical approval was obtained from the University of Northampton Graduate School Research Ethics Committee.

6.2.2. Study Design

Participants performed a 6 week exercise training programme incorporating a mixture of endurance and strength training, with intensity increasing after three weeks. An

independent groups design was employed where WBC participants performed their programmes in conjunction with two weekly WBC treatments. The control (CON) group underwent the same training programme without cryotherapy intervention. To assess the impact of the training, pre and post tests were conducted on physiological, performance and anthropometric characteristics. The study design is summarised in Figure 6.2.2.

Table 6.2.1: Summary of characteristics for whole body cryotherapy (WBC) and control (CON) participants in the training study. Data presented as means ± standard deviations.

	WBC (n=7)	**CON (n=9)**	**OVERALL (n=16)**	**T Test between groups**
Age (yrs)	38.0 ± 10.0	29.8 ± 8.5	33.4 ± 9.8	p=0.10
Height (m)	1.80 ± 0.04	1.79 ± 0.06	1.79 ± 0.05	p=0.64
Body mass (kg)	80.7 ± 7.7	83.6 ± 11.5	82.3 ± 9.8	p=0.58
Body mass index (kg/m^2)	24.9 ± 2.2	26.1 ± 3.5	25.6 ± 3.0	p=0.42
Body fat %	20.4 ± 5.5	19.4 ± 5.3	19.8 ± 5.3	p=0.74
Absolute VO_2 max (l/min)	3.75 ± 0.38	3.59 ± 0.53	3.66 ± 0.46	p=0.53
Relative VO_2 max (ml/min/kg)	46.7 ± 5.4	43.2 ± 5.6	44.8 ± 5.6	p=0.23

6.2.3. Experimental Procedures – Pre and Post-test

All participants were asked to refrain from caffeine, alcohol and strenuous exercise for 4, 24 and 48 hours respectively prior to testing sessions. Upon arriving for the first test, participants' blood pressure was assessed in a seated position. They were then asked to lie on a massage bed in a supine position for the assessment of their right femoral artery blood flow by Doppler ultrasound as described previously (section 3.2). Following this, anthropometric characteristics were assessed, including height and body mass. A skinfold assessment for body fat percentage estimation was conducted according to ISAK guidelines, as explained previously (section 3.2).

Participants were familiarised to the muscle torque assessment using the Biodex dynamometer, with the same procedure followed as in the previous studies (sections 3.2, 4.2 and 5.2). Participants then prepared for their VO_2 max assessment on the treadmill. This involved a brief warm up and the attachment of a face mask linked to the gas analyser for the direct assessment of oxygen consumption levels. The same protocol was followed as explained previously (sections 3.2, 4.2 and 5.2) with participants exercising incrementally until volitional exhaustion. Following test completion, participants were allowed fluids and a cool down and recovery period. The absolute and relative VO_2 max values were reported and 70% and 75% of the absolute VO_2 max were calculated.

Once fully recovered, participants underwent a barbell squat test where the maximum weight lifted for three repetitions was determined (Haff & Triplett, 2016). Due to adverse reactions reported from participants in a pilot study examining one repetition maximum performance (section 3.6), three repetitions was deemed safer and more appropriate for this study. Participants performed 6-10 warm-up repetitions with a 20kg Olympic barbell, ensuring appropriate technique (Myer *et al.,* 2014), followed by one set of 6 repetitions at a weight equivalent to between 50-75% of the participant's body mass. The weight was then gradually increased by the addition of weight plates (Eleiko, Sweden) until participants attained the maximum weight they could complete the movement with correct form for three repetitions. The weight increments ranged from 2.5kg to 10kg, depending on the perceived effort of the participant. Participants were given at least a two minute recovery period between each attempt. Participants were required to descend until their quadriceps were parallel to the ground and to fully extend at the knees on the upward phase. Proper activation of the gluteal muscles was also encouraged to ensure movement efficiency and reduced risk of muscle imbalances and injuries (Myer *et al.,* 2014). Verbal encouragement and close supervision was ensured throughout. The three repetition maximum (3RM) weight was converted to one repetition maximum (1RM) using a conversion calculator.

Unilateral isometric maximal muscle torque of the right quadriceps was assessed on the Biodex dynamometer. The procedure followed was similar as described previously (section 3.2), except there was one submaximal contraction and three maximal contractions (with two minute recoveries) with the highest torque produced determined as the individuals pre-test score (Baross *et al.,* 2013).

The final exercise test was a maximal countermovement jump (CMJ) for the assessment of explosive power. Participants were required to vertically jump on a mat (Perform Better, Southam, UK), aiming for maximum height 5 times whilst keeping their legs extended and hands on hips prior to landing (Figure 6.2.1). Participants were also asked to land in the same position each jump (Markovic *et al.,* 2004). The flight times (ms) and heights (mm) were measured for each jump. Due to the presence of microswitches embedded in the mat, flight time was measured as the interval between lift-off and landing of the feet and automatically converted into jump height. The means from the highest three jumps were recorded as the individuals pre-test score (Young *et al.,* 1995). The CMJ has been demonstrated to be a reliable test for jump power, effectively activating the stretch-shortening cycle and associating closely with sports performance parameters such as power, speed and agility (Markovic *et al.,* 2004; Rodriguez-Rosell *et al.,* 2017; Van Hooren & Zolotarjova, 2017)

Participants returned to the laboratory 3-7 days following their final training session to have the same six tests re-assessed in the same order. Caffeine, alcohol and strenuous activity were avoided as before and the post-test was conducted at a similar time of day as the pre-test. The same procedures (except for muscle torque familiarisation) were followed to determine each participant's post-test score.

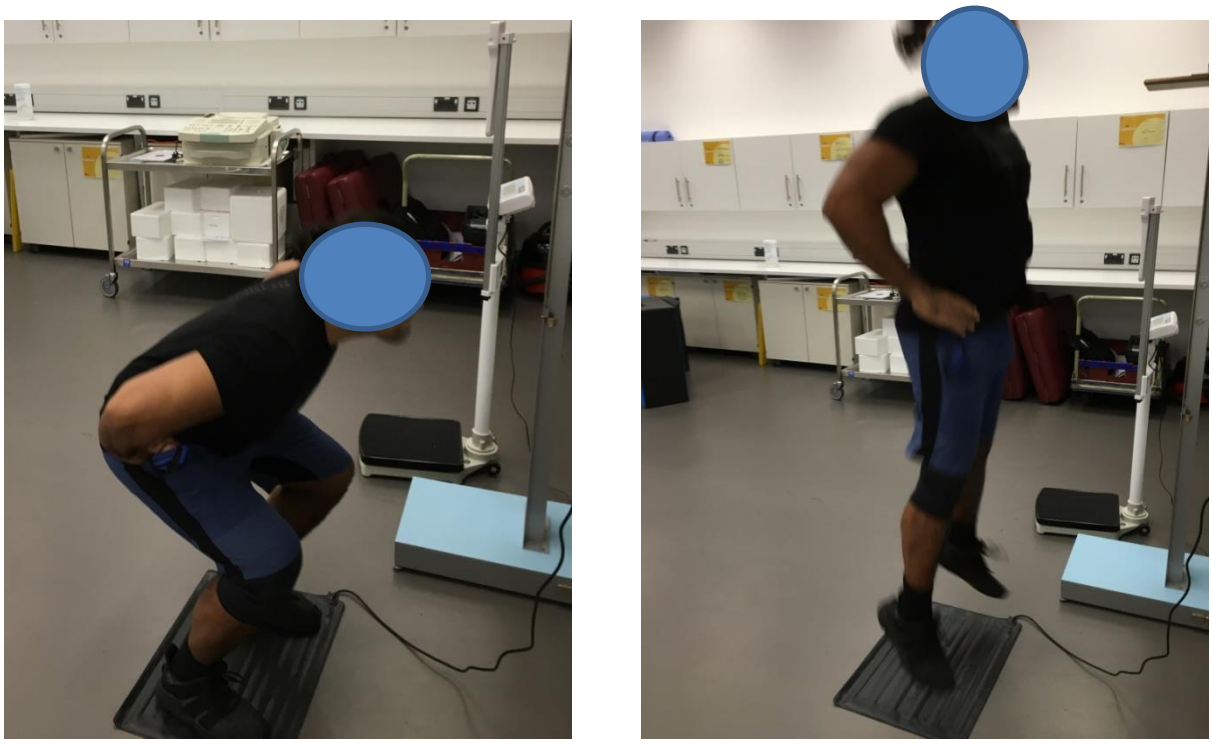


Figure 6.2.1: Participant performing the countermovement jump test.

6.2.4. Training Programme and Exercises.

A six week training duration was considered an appropriate compromise between attaining significant adaptations (Kraemer *et al.,* 1998; Marrier *et al.,* 2018) and timing/logistical constraints (e.g. use of the cryotherapy chamber). Participants commenced their training programme between 3-7 days following their pre-test. The programme consisted of four sessions per week: two 30 minute treadmill runs at 1% incline and initial intensity equivalent to 70% VO_2 max, as well as two strength and plyometric training sessions involving lower body exercises with two minute recoveries between sets. There were five exercises:

- Barbell squats – 4 sets of 6 repetitions at 70% estimated 1RM

- Dumbbell lunges – 3 x 8 repetitions on each leg

- Nordic leg curls - 2 x 8

- Depth jumps – 3 x 8 from a step/box height of 30cm

- Split lunge jumps – 3 x 8 on each leg with hands on hips

Participants were familiarised with all exercises beforehand ensuring capability of performing sessions autonomously. The four sessions were performed on separate days of the week in the order of run>weights>run>weights. Since many of the participants were previously untrained, a frequency of four total sessions was considered an appropriate balance between attaining adaptations and preventing overtraining and/or injury. The WBC participants received cryotherapy treatment within 30 minutes following completion of their first and final training sessions each week. The same WBC protocol was followed as explained previously (section 3.3).

After three weeks, the treadmill speed progressed to an intensity corresponding to 75% of initial VO_2 max and the barbell squat weight increased to 75% of estimated 1RM to ensure appropriate progressive overload. Participants were instructed to continue with their usual diets and activity levels outside of the prescribed sessions.

6.2.5 Verification of training running speeds

Following completion of the pre-tests and sufficient recovery, participants were linked to the gas analyser via a face mask to determine their prescribed treadmill running

speeds. For the first three weeks of the programme, participants were required to run at a speed eliciting an intensity equivalent to 70% of absolute VO_2 max. The intensity progressed to 75% VO_2 max for the second three weeks. Participants ran on the treadmill (1% incline) at a steady speed with VO_2 values continuously monitored. The speed was adjusted slightly until a VO_2 value equivalent to 70% VO_2 max was attained on a consistent basis. Expired gas data was averaged over a period of at least a minute of running at a constant speed during this process. Following establishment of the speed at 70%, the speed was progressed slightly and the same process was applied for determination of the speed at 75% VO_2 max.

6.2.6. Statistical Analysis

All data was analysed using SPSS Version 26. Data for all variables was assessed for normal distribution by the Shapiro-Wilk test and extent of skewness and kurtosis. There was no significant deviation from normality in any of the variables except for 3RM squat, where a log transformation was applied. A two way repeated measures ANOVA was used to assess the interaction effect between treatment (WBC vs. CON) and time for all six outcome variables. Paired t tests were applied to examine pre-post differences within the WBC and CON groups. Significance levels were set at 0.05.

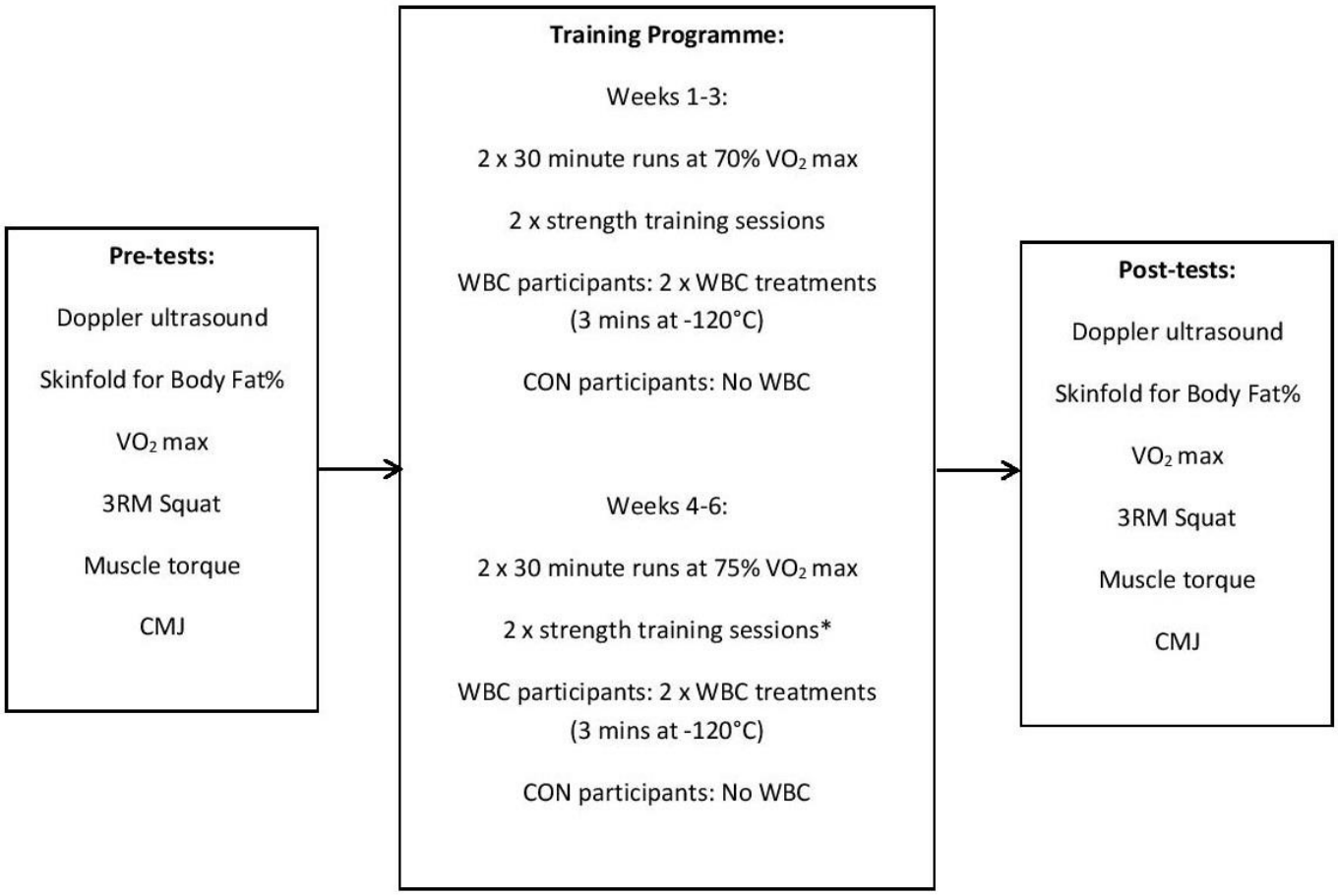


Figure 6.2.2: Summary of design for training study with 4 sessions performed weekly. WBC – Whole Body Cryotherapy; CON – Control; 3RM – 3 repetition maximum; CMJ – countermovement jump test. * weight for barbell squat progressed from 70% 1RM to 75% 1RM after 3 weeks.

6.3. Results

Body Fat Percentage

Body fat percentage significantly decreased following the 6 week training programme for the CON group (19.4 ± 5.3 % vs. 18.6 ± 5.1 %, p=0.01) whilst the decrease approached significance for the WBC group (20.4 ± 5.5 % vs. 19.6 ± 5.9 %, p=0.08, Figure 6.3.1). There was no difference between groups over time (interaction effect, p=0.9). There was no difference in body mass before and after the training for either group (WBC, 80.7 ± 7.7 kg vs. 80.7 ± 9.3 kg, p=1.0; CON, 83.6 ± 11.5 kg vs. 84.2 ± 10.6 kg, p=0.24)

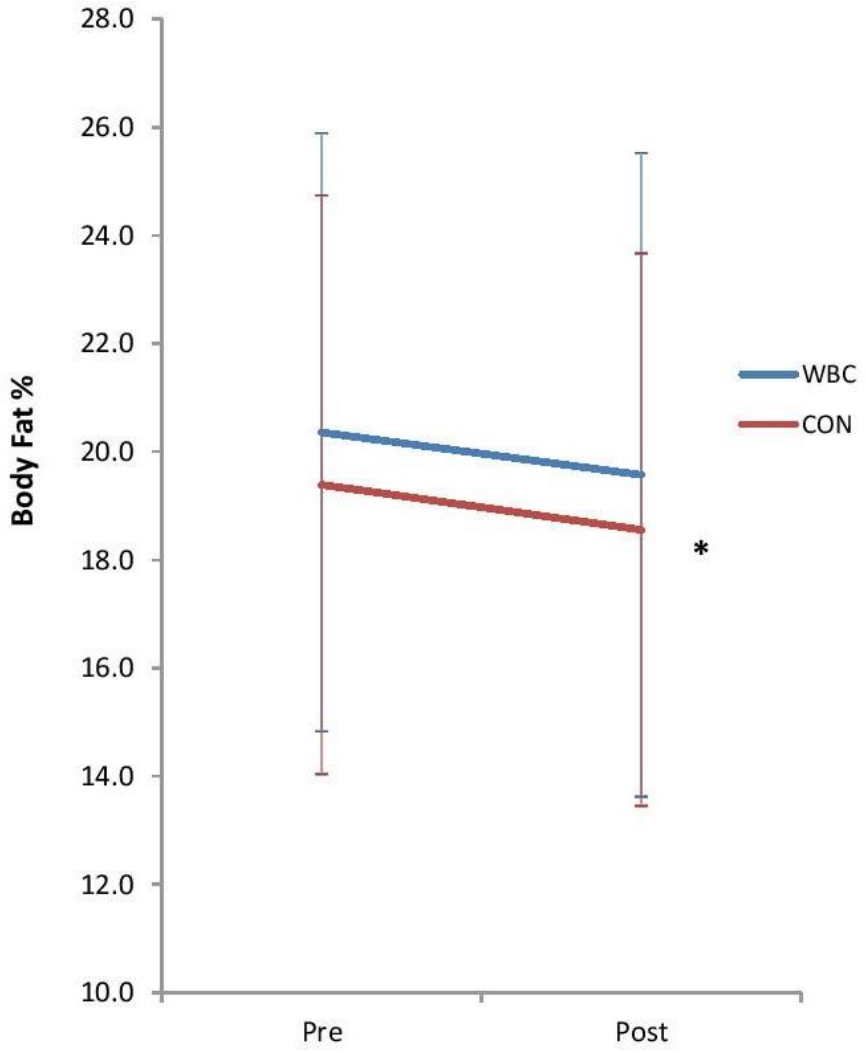


Figure 6.3.1: Body fat percentages before and after the 6 week training programme for WBC and CON groups. *p<0.05 for body fat decrease in CON. Data presented as means ± standard deviations. N=16.

Relative VO_2 Max

There was no significant increase in relative VO_2 max following the training programme for either WBC (46.7 ± 5.4 ml/min/kg vs. 47.9 ± 4.9 ml/min/kg, p=0.25) or CON (43.2 ± 5.6 ml/min/kg vs. 44.3 ± 5.1 ml/min/kg, p=0.24, Figure 6.3.2). There was no interaction between treatment and time (p=0.9).

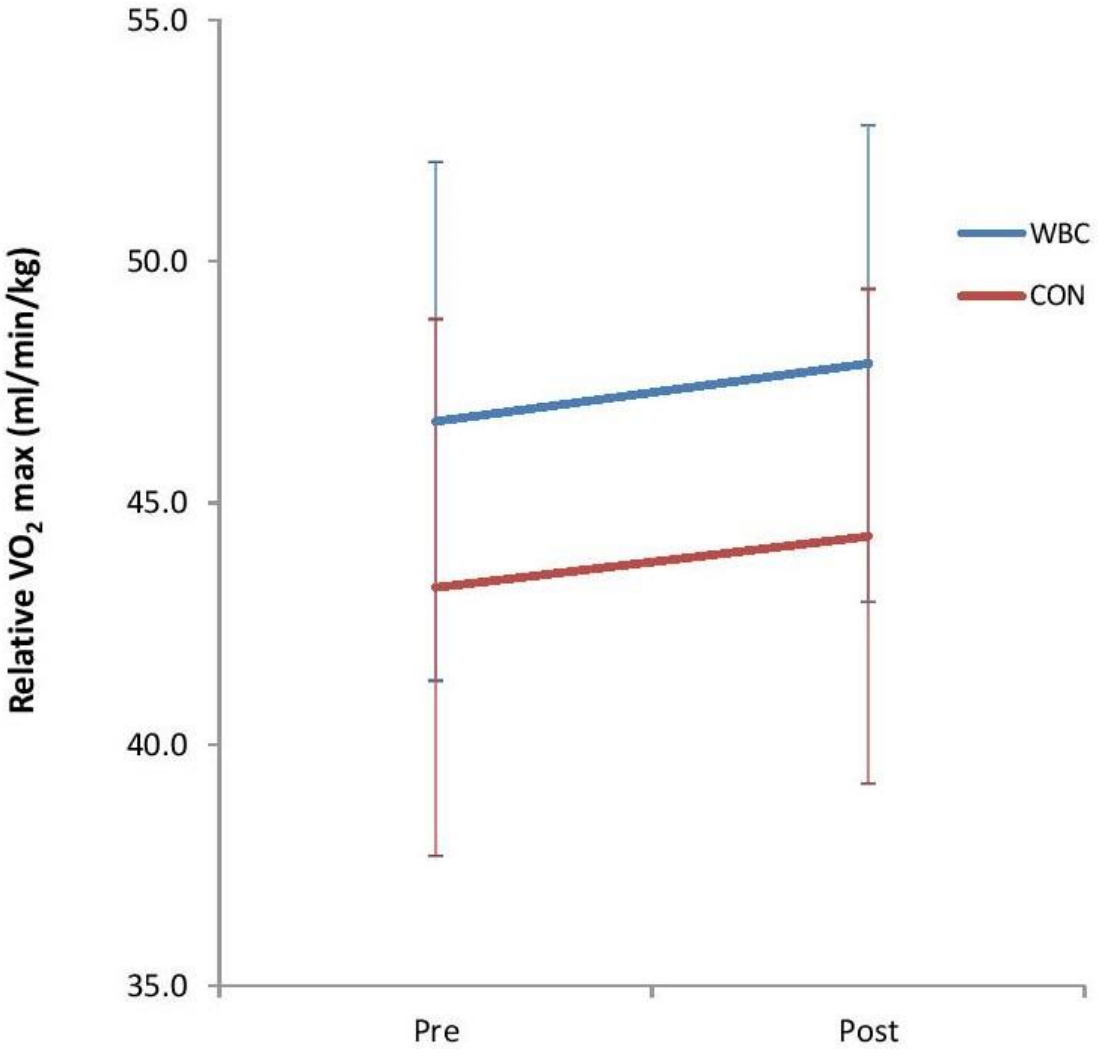


Figure 6.3.2: Relative VO_2 max for WBC and CON groups before and after 6 week training programme. Data presented as means ± standard deviations. N=16.

Muscle Torque

Maximal isometric leg muscle torque significantly increased following the training in both WBC and CON groups (WBC, 277.1 ± 63.2 Nm vs. 318.1 ± 83.4 Nm, p=0.00; CON, 244.6 ± 50.6 Nm vs. 268.0 ± 71.8 Nm, p=0.05, Figure 6.3.3). There was no significant difference between groups over time (interaction effect, p=0.21).

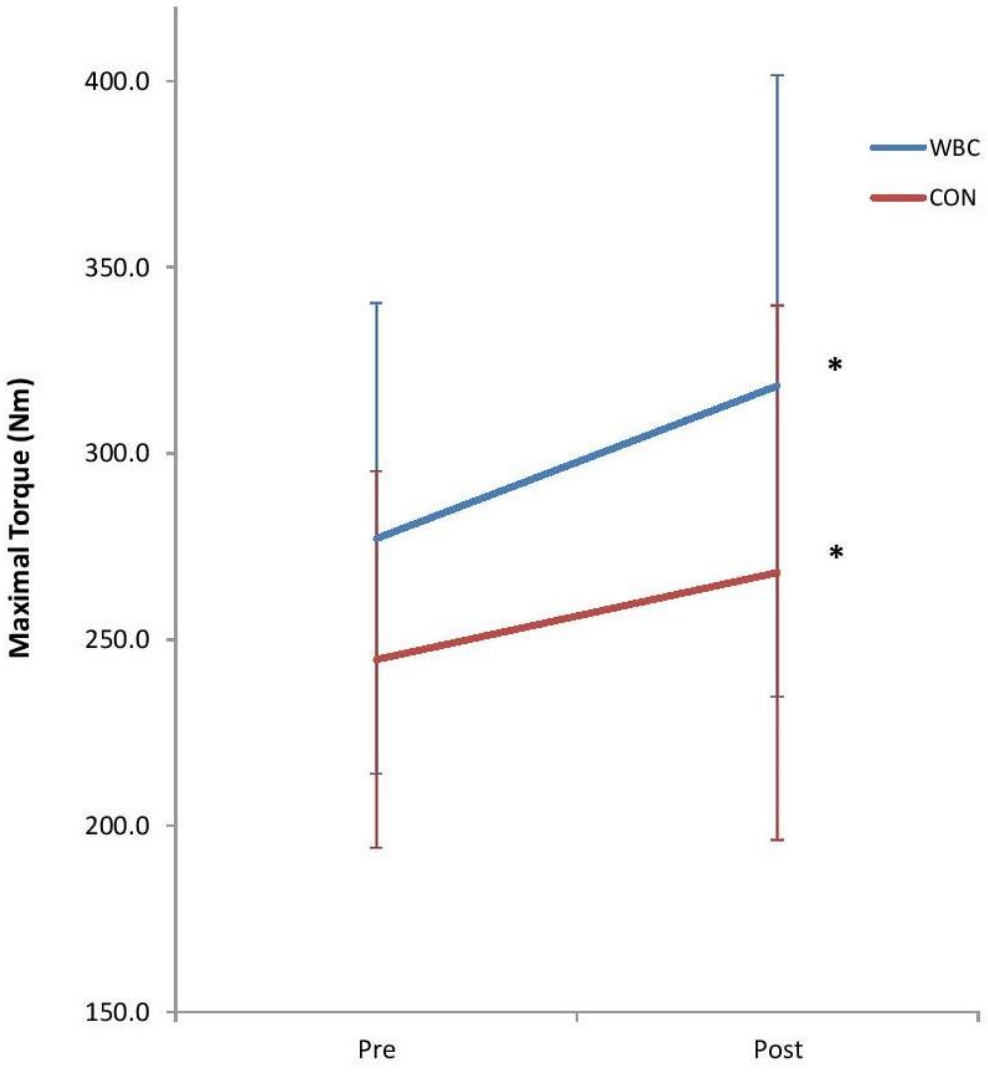


Figure 6.3.3: Leg muscle torques for WBC and CON before and after 6 week training programme. *p≤0.05 for increase in both groups. Data presented as means ± standard deviations. N=16.

Barbell Squat

Three repetition maximum squat significantly increased in both groups following the training (WBC, 86.4 ± 19.5 kg vs. 98.9 ± 15.2 kg, p=0.03; CON, 91.1 ± 28.7 kg vs. 106.1 ± 30.0 kg, p=0.00, Figure 6.3.4). There was no difference between groups over time (interaction effect, p=0.8).

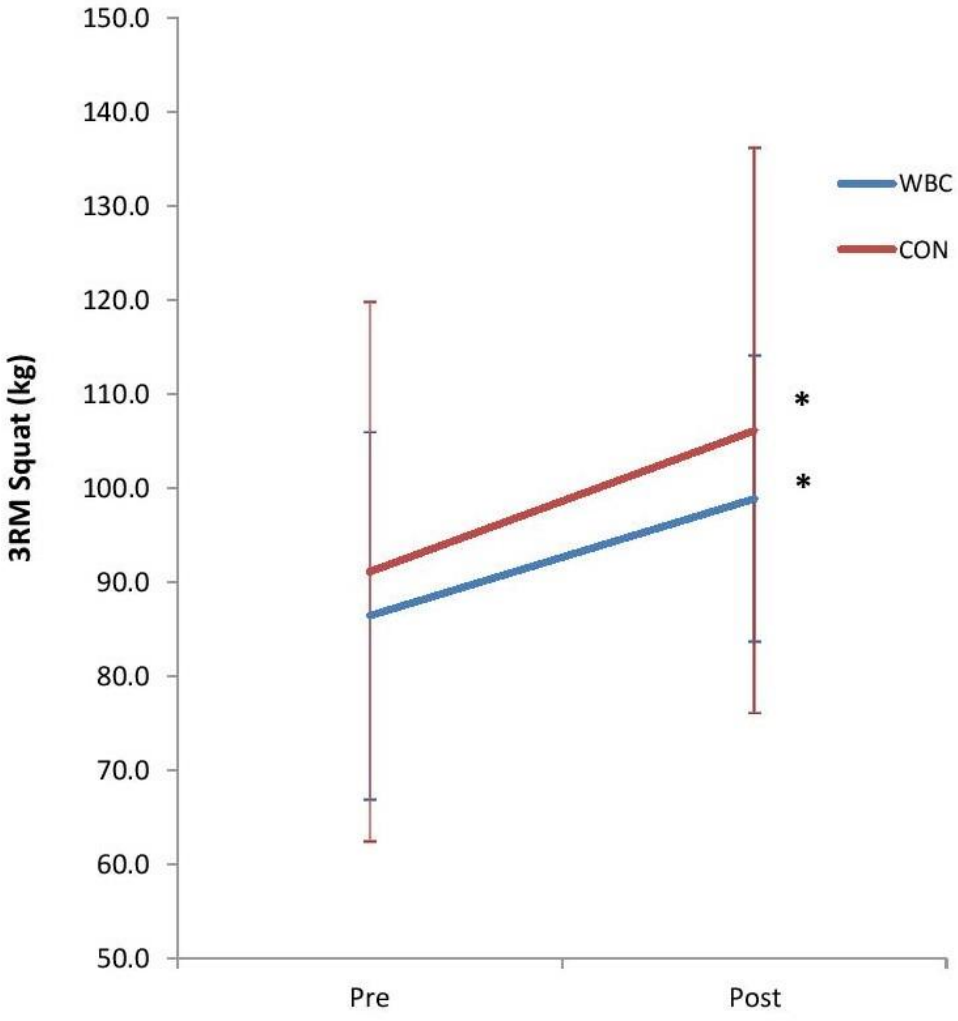


Figure 6.3.4: Barbell squat three repetition maximums for WBC and CON before and after 6 week training programme. *p≤0.05 for increase in both groups. Data presented as means ± standard deviations. N=16.

Doppler Ultrasound

There was no difference in femoral artery blood flow for either WBC (230.1 ± 52.2 ml/min vs. 262.2 ± 133.5 ml/min, p=0.56) or CON (270.5 ± 96.0 ml/min vs. 300.1 ± 86.8 ml/min, p=0.35). There was no interaction between group and time (p=0.97).

Countermovement Jump

Jump height did not significantly increase in the WBC group following the training programme (302.3 ± 44.0 mm vs. 312.3 ± 47.8 mm, p=0.23), whilst there was a significant increase for the CON group (293.3 ± 45.2 mm vs. 328.1 ± 69.2 mm, p=0.01, Figure 6.3.5). The overall interaction between group and time approached significance (p=0.07).

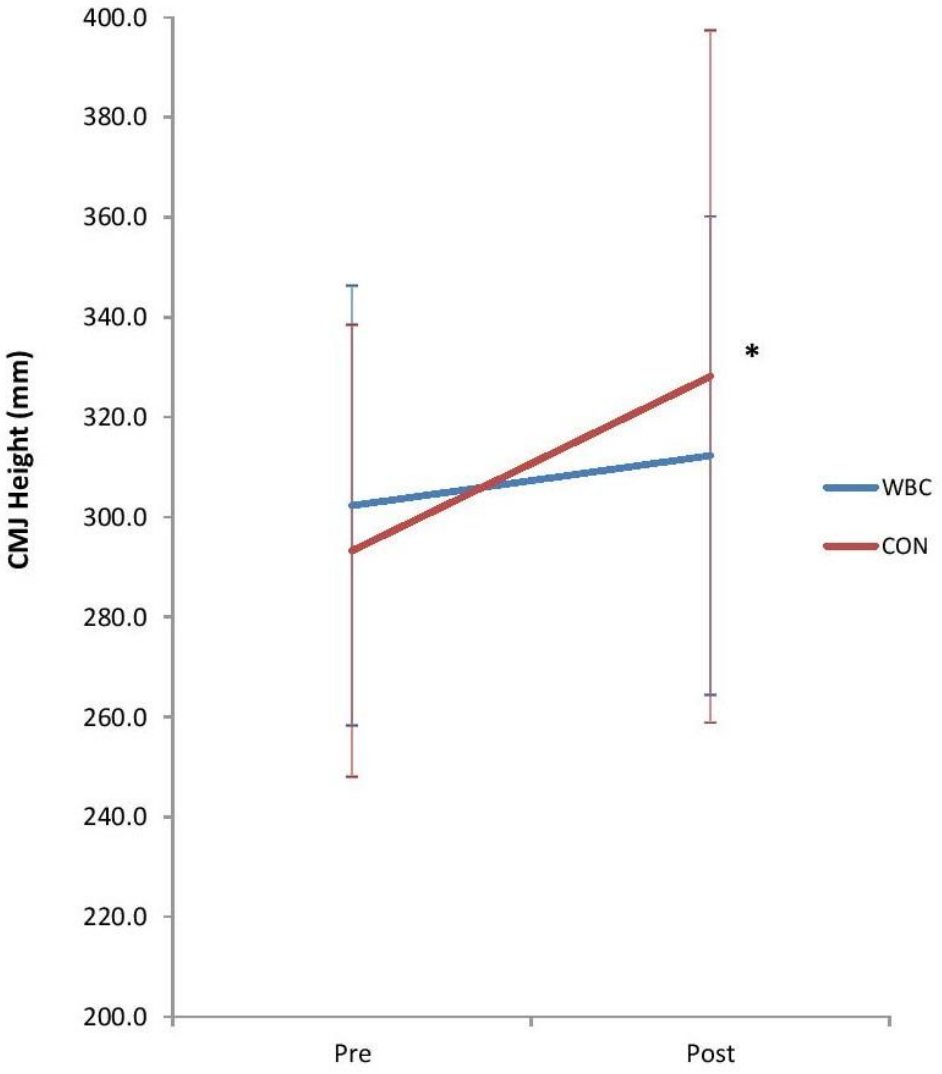


Figure 6.3.5: Countermovement jump height for WBC and CON before and after 6 week training programme. *p<0.05 for increase in CON group. Data presented as means ± standard deviations. N=16.

6.4. Discussion

The 6 week concurrent and progressive training programme was generally effective in improving lower body strength, jump power and body composition. However, there were no significant gains in aerobic endurance or arterial blood flow. Repetitive whole body cryotherapy did not appear to have a detrimental impact on such fitness attributes, however it might hinder improvements in countermovement jump power.

Prior studies on repetitive CWI treatments (Fuchs *et al.,* 2020; Roberts *et al.,* 2015) indicate that repetitive cryotherapy is unfavourable and potentially damaging to long term training adaptations. This could be partly due to dampening of the inflammatory response which would otherwise be a necessary component of adaptive gains (White & Wells, 2013). This current study indicates that repetitive cryotherapy does not hinder such adaptations, particularly with regards to muscle strength. However, the lack of significant increase in countermovement jump height for the cryotherapy group indicates that repetitive WBC may hinder adaptations in explosive power which would be of concern to athletes where power is an important performance component.

This is one of the first studies to investigate the impact of repetitive WBC treatment on adaptations to a strength and endurance training programme. Previous studies that have examined the influence of WBC treatments on training responses adopted training of a single type – interval (Broatch *et al.,* 2019) or resistance (Jaworska *et al.,* 2020) or were a much shorter duration of just two weeks (Jaworska *et al.,* 2018; Jaworska *et al.,* 2021). The inclusion of a mixture of endurance, strength and power training in this study is arguably more representative of sports training programmes. The 24 training sessions performed in this programme is also substantially more than the aforementioned studies. It is therefore likely that the overall higher load in this 6 week programme would induce more significant adaptations.

The training programme was effective in improving the participants' body compositions following the 6 week programme, as evidenced by the significant decreases in body fat percentages assessed via skinfold (Figure 6.3.1). Since there was no difference in body mass before and after the programme, it is highly probable that the participants increased their lean muscle mass. Increases in lean body mass is a common observation following exercise training programmes, particularly when

the exercises are unaccustomed (Benito *et al.,* 2020, Brook *et al.,* 2015). Repetitive WBC treatments did not blunt this adaptive response (0.8% decrease in body fat for both WBC and CON groups). Therefore sports competitors and practitioners do not need to be concerned about any potential detrimental impact of such treatments on body composition following a training programme.

The outcomes of this study would suggest that it is unlikely that WBC treatment can be used as an effective tool to reduce body fat and improve body composition in conjunction with an athletic training programme. However, results may differ with a higher frequency of weekly WBC treatments. The relationship between body composition and sports performance has already been well documented (Collins *et al.,* 2014; Esco *et al.,* 2018; Mattila *et al.,* 2007). It is theorised that cold treatments could support changes in body composition via increased thermogenesis, activity of brown adipose tissue and subsequent caloric burn and fat loss (Cannon & Nedergaard, 2012). Pilch *et al.* (2020) recently demonstrated fat mass decreases following repetitive WBC, however no experimental study has demonstrated promising results regarding repetitive WBC and body fat content in a sports or training context.

There were clear improvements in muscle strength following the training programme, as assessed by both barbell squat performance and maximal isometric muscle torque (Figures 6.3.3 and 6.3.4). Numerous studies have documented the relationship between lower body strength and sports performance (Seitz *et al.,* 2014) as well as the benefits and popularity of the barbell squat exercise in sports training programmes (Clark *et al.,* 2012; Vecchio, 2018). The improvements in barbell squat three repetition maximum and muscle torque were comparable in both WBC and CON groups, indicating that repetitive cryotherapy treatment does not attenuate the adaptive gains of muscular strength following a strength training programme. Although this study did not assess protein synthesis rates or muscle fibre cross sectional areas, it is conceivable that due to the likely increases in lean body mass, the strength gains in both groups were the result of a combination of muscle hypertrophy, neurological adaptations and superior motor unit recruitment (Del Vecchio *et al.,* 2019; Haff & Triplett, 2016).

The outcome of the lack of difference in strength response between WBC and CON groups contradicts previous findings of repetitive CWI attenuating gains in muscular strength (Roberts *et al.,* 2015). The fairly modest quantity of 12 cryotherapy

treatments in total could be a factor in the lack of hindrance effect on adaptations. An alternative explanation is that the modality and mechanisms of the cold exposure in WBC is different to that of CWI (Bleakley *et al.,* 2014). Cold air has lower thermal conductivity than cold water and may therefore not hinder subsequent amino acid supply to muscles and anabolic cell signalling. This potential explanation is supported by findings that CWI elicits more pronounced reductions in lower limb blood flow than WBC (Mawhinney *et al.,* 2017). Further studies on repetitive WBC treatment in training programmes are necessary to form conclusions regarding the potential impact and mechanisms of repetitive cold exposures on attenuating gains in muscle strength/hypertrophy.

The 6 week training programme was not effective in improving aerobic endurance, since VO_2 max did not significantly increase in either the WBC or CON group. One possible explanation for this is the relatively low aerobic training volume and/or intensity, with two steady 30 minute runs at a moderate intensity of 70-75% VO_2 max. Since the participants were not aerobically highly trained (with fairly modest aerobic capacities at the start of the programme), it was expected that two steady state runs a week would be sufficient to elicit improvements. Another possibility is that potential gains were hindered by the 'interference effect', as explained in section 6.1. Since two weekly sessions of weight training were also performed in the programme, it is plausible that the mechanisms in which substantial adaptations in muscle strength were elicited had an interference effect on the mechanisms/pathways of adaptations to aerobic endurance. As mentioned prior (section 6.1), the adaptive pathways for endurance and strength training are independent and not compatible with each other, with the interference effect from concurrent training methods being previously demonstrated (Coffey & Hawley, 2017). This highlights the difficult dilemma that many athletes face when structuring their training programmes aiming to enhance both endurance and strength.

The training programme did not induce any gains in arterial blood flows. The lack of improvement in arterial blood flow may be due to several reasons. Firstly, two endurance sessions a week may not be sufficient volume to induce benefits, as was probably the case for the lack of improvement in aerobic endurance. However, lower body resistance exercise programmes are expected to result in increased arterial blood flow (Anton *et al.,* 2006; Tanimoto *et al.,* 2009) which would rule out any possible interference of strength training on arterial blood flow gains. Alternative

reasons for the lack of arterial blood flow increase are the relatively short duration of the training programme as well as the fitness levels of the participants. Previous studies that have demonstrated significant training-induced improvements in lower limb arterial blood flow typically used sedentary and/or older participants (Anton *et al.,* 2006; Collier *et al.,* 2008). The participants in this study (whilst not necessarily trained athletes) were mostly young and reasonably active and may therefore not be ideal candidates for inducing substantial gains in arterial blood flow. No prior study had investigated the impact of repetitive WBC on resting arterial blood responses following exercise training. Thus, further research is required in this area to elucidate the role that WBC might have in mediating blood flow adaptations.

Whilst WBC has previously shown to benefit muscle power in the short term (Fonda & Sarabon, 2013; Lubkowkska, 2012) and could support post-activation potentiation following exercise (Partridge *et al.,* 2019), the impact of repetitive WBC on long term muscle power development remains inconclusive. It was the intention of the researcher to include power specific exercises to take advantage of activating the stretch-shortening cycle. This inclusion would more likely be representative of sports training programmes. For instance, the depth jump exercise has previously been demonstrated to be effective in eliciting power improvements following a 6 week programme (Mclenton *et al.,* 2008). Countermovement jump height significantly improved in the CON group (293.3 mm vs. 328.1 mm), indicating that the programme was suitable for training leg power. However, the cryotherapy group did not demonstrate such improvements (Figure 6.3.5). This might indicate that repetitive WBC blunts the adaptive response to gains in power. The possible physiological mechanisms for this hindrance are not clear, particularly when the cryotherapy group significantly improved their muscle strength, which is an important component of power (Kraemer & Looney, 2012). Whilst the total force output was unaffected, the rate of force development could have been compromised by repetitive WBC applications. Possible mechanisms for this attenuation could be a reduction in motor unit activation and/or signalling frequency from the central nervous system. The cryotherapy participants did not appear to improve their stretch-shortening cycle capabilities, potentially compromising the elastic properties of the muscle despite improvements in total force capabilities (Kraemer & Looney, 2012). The possible mechanisms in which repetitive cold could compromise the rate of force development is an area that requires further research before conclusions can be drawn regarding the effects.

Study Limitations

The relatively small sample size for the cryotherapy group should be taken into account before interpreting the results, particularly in concluding that repetitive cryotherapy blunts adaptations in muscular power. Whilst it is highly unlikely that a larger sample would substantially alter the outcomes regarding strength, VO_2 max and arterial blood flow (due to similar responses to the CON group), it is conceivable that the outcome in jump height was impacted by this sub-optimal sample.

Another potential factor is that the lack of impact of repetitive cryotherapy treatments on adaptive responses to training may be due to the relatively low number of treatments. Higher frequencies of WBC treatments are more likely to significantly attenuate adaptive inflammatory responses (Zembron-Lacny *et al.,* 2020). In this study, logistical and timing constraints meant that it was not feasible for each participant to perform more than two weekly cryotherapy treatments. Additionally, the economic viability of higher numbers of weekly WBC treatments requires further consideration.

Conclusions

In conclusion, repetitive whole body cryotherapy treatment did not have a significant negative impact on adaptations to muscle strength or body composition following a concurrent 6 week training programme. Sports practitioners can cautiously apply repetitive WBC to support recovery post-exercise without undue concern on athletes' fitness gains or long term performance; however the potential negative impact on muscular power remains inconclusive. Further research should focus on the potential mechanisms by which repetitive WBC can affect physiological adaptations to sports training programmes, particularly with regards to muscle power and blood flow. Furthermore, the economic implications of higher frequencies of cryotherapy treatments require further review.

CHAPTER 7 –

OVERALL DISCUSSION

This chapter will synthesise the findings and significance of the three main studies implemented in this thesis to further evaluate the effects of Whole Body Cryotherapy (WBC) treatment for sports recovery and performance. Specific mechanisms and theories will be covered, in light of previous research. Additionally, individual sections will discuss some important issues regarding the use of WBC, specifically inter-individual differences, optimal treatment protocol factors and comparisons with cold water immersions (CWI), as highlighted previously in chapter 2. Finally, potential implications for sport and exercise practice as well as future research will be considered, before summarising the main conclusions in relation to the objectives specified in chapter 1.

7.1. General Synthesis of Studies and Mechanisms

Drawing the findings from the first two main studies, it can be concluded that a single treatment of WBC taken one hour post-exercise alleviates the muscle strength reduction that is evident following a downhill run. This supports the use of WBC for post-exercise recovery. This muscle strength outcome was significantly influenced by age. Young males in particular can benefit with single WBC treatments one hour after eccentrically biased exercise to support recovery. Despite this positive effect, WBC was no more effective for post-exercise recovery than CWI, which means that the cost vs. reward implications of WBC treatments would benefit from further evaluation. The final study revealed that repetitive WBC did not have an overall negative impact on training adaptations, supporting its use in concurrent training programming and sports periodization; however there may be an interference effect on the development of explosive power.

One aim of this thesis was to evaluate the effect of WBC treatment for promoting sports recovery via its potential to treat exercise-induced muscle damage (EIMD). As discussed previously (section 2.3.1) and in several comprehensive reviews (Hody *et al.,* 2019; Peake *et al.,* 2017), EIMD is commonly observed following exercise, particularly those involving extensive eccentric contractions. Whilst different EIMD protocols have been utilised to assess the effectiveness of post-exercise recovery interventions, one focus of this thesis was to evaluate the impact of WBC following a downhill run. As discussed earlier (sections 3.6 and 4.1), downhill running remains an under-investigated exercise model in the context of WBC, despite the availability of numerous downhill run studies observing common EIMD markers (Dolci *et al.,* 2015;

Fortes *et al.,* 2013; Malm *et al.,* 2004). Since the overall effectiveness of WBC for treating muscle damage is equivocal due to mixed findings (Fonda & Sarabon, 2013; Hausswirth *et al.,* 2013; Hohenauer *et al.,* 2017; Vieira *et al.,* 2015), it was beneficial to explore further the potential efficacy of WBC for treating muscle damage following a different exercise protocol. The discrepant findings on the effects of WBC for post-exercise recovery could be partially explained by the specific mechanisms of EIMD. Downhill running is a whole body multi-joint exercise, which is likely to cause less severe mechanical strain than other damage protocols involving strenuous isolated contractions (Fatouros & Jamurtas, 2016; Paulsen *et al.,* 2012; Peake *et al.,* 2017). This was demonstrated in the current body of work, since the muscle torque decrement (although significant) was only 11-12% for the CON groups in the first two main studies (chapters 4 and 5) and the average peak plasma creatine kinase (CK) was <600IU/L - substantially lower than other muscle damage studies (Paulsen *et al.,* 2010; Yoon & Kim, 2020) .

Although not measured directly, significant leukocyte infiltration may not have occurred in these studies due to the mild damage and lack of significant alterations of IL-6, indicating a lack of acute-phase inflammatory response. Therefore, it is evident that the 30 minute downhill run at 15% decline caused substantial yet mild muscle damage and the extent of torque decrease was not as severe as what would be classified as moderate or severe muscle damage (Paulsen *et al.,* 2012). Nonetheless, the significant presence of muscle soreness and CK implies that several of the EIMD mechanisms previously reported were also present in the first two main studies, notably Z-line streaming, myofibrillar disruption, disturbance of calcium homeostasis and failure of excitation-contraction coupling mechanisms (Owens *et al.,* 2018).

An independent groups design utilising separate control groups was deemed appropriate to investigate the effect of WBC post-exercise to avoid any possibility of the repeated bout effect (RBE) from crossover designs. The RBE has been demonstrated in previous downhill run studies (Byrnes *et al.,* 1985; Dolci *et al.,* 2015; Smith *et al.,* 2009), whereby the muscle damage is significantly less pronounced following the second bout due to muscular adaptations. Eccentric contractions can therefore be promoted as an effective means of promoting muscular strength due to the remodelling mechanisms mediated by morphological and metabolic adaptations (Hody *et al.,* 2019). This protective effect has been reported to last up to 6 months

(Nosaka *et al.,* 2001). Thus, it was considered necessary to avoid any training effect in the cohort of participants undergoing the downhill runs in this body of work. Since one of the purported benefits of cryotherapy is to reduce muscle breakdown post-exercise, thereby promoting quicker recovery (Ferreira-Junior *et al.,* 2014), part of the rationale for undergoing the training study (chapter 6) was to investigate the possibility of repetitive WBC having an interference effect on this adaptive response. This does not appear to be the case, since repetitive WBC participants developed their muscle strength comparably to their control counterparts. As mentioned in chapter 6, the muscle strength improvements following the training programme may have been due to a combination of neural adaptations and muscular hypertrophy, thus repetitive WBC does not appear to negatively impact such mechanisms.

The responses of some of the variables assessed in this thesis are consistent with the previously established physiological effects of WBC treatment, notably substantial reductions in core and skin temperatures. The core temperature reduction in the first main study was statistically significant, however the implications of this response on subsequent exercise performance is open to interpretation. It has been reported than reductions in body heat strain can reduce fatigue and promote exercise recovery (Marino 2002) and reductions in core temperature post-WBC have been associated with enhanced subsequent endurance performance (Kruger *et al.,* 2015). This would appear advantageous for athletes, however it is not clear to what extent this would benefit recovery 24 hours post-exercise. The skin temperature response observed in the first and second main studies (chapters 4 and 5) are consistent with previous findings (Costello *et al.,* 2012; Cuttell *et al.,* 2017) and the notion that extreme cold causes a pronounced cutaneous vasoconstriction response due to the excitation of sympathetic α-adrenergic fibres (Bouzigon *et al.,* 2016). Following this, there is a rebound vasodilatory effect which can affect central blood volume, further mediating the recovery response to exercise via extra oxygen supply and removal of waste products (White & Wells, 2013).

Regarding muscle recovery, it is not clear why WBC would alleviate muscle strength reduction whilst failing to alleviate muscle soreness or plasma CK. For instance, studies indicate a lack of support for WBC having a stimulatory effect on satellite cells for muscle regeneration post-exercise (Vieira Ramos *et al.,* 2016; Zembron-Lacny *et al.,* 2020). Different theories exist in the literature regarding the mechanisms by which WBC can treat EIMD. An alleviation of sICAM-1 (an established mediator

molecule of muscle inflammation and leukocyte adhesion) curtailing further muscle breakdown was proposed by Ferreira-Junior *et al.* (2014). However, recent findings have contradicted this theory by discovering that single WBC was ineffective in altering siCAM-1 levels or inflammatory markers post-exercise (Krueger *et al.,* 2018). Whilst anti-inflammatory responses have been observed previously following WBC (Pournot *et al.,* 2011), the downhill run in this project did not induce a marked increase in plasma IL-6 levels (chapter 5). Therefore it remains inconclusive what the precise impact of WBC is on IL-6 levels and difficult to evaluate how WBC can enhance muscle recovery via anti-inflammatory properties. Additional possibilities for the benefits of WBC post-exercise are increased anti-oxidant defence (Miller 2012; Zembron-Lacny *et al.,* 2020), curtailed generation of reactive oxygen species (White & Wells, 2013) and stabilisation of lysosomal enzymes (Wozniak *et al.,* 2009) which could collectively contribute to mitigating myofibrillar disruption and retaining muscle contractile properties post-EIMD.

Based on the findings of this thesis, it would be difficult to conclude that WBC can mediate muscle recovery via alterations in blood flow due to the lack of decrease in femoral artery blood flow in main study 2 (chapter 5). Reductions in muscle blood flow and metabolism is a proposed mechanism for the potential alleviation of muscle damage and subsequent recovery, owing to reduced cell death and secondary damage (White & Wells, 2013). Since it has been argued that muscle strength is the most important marker of muscle function and thereby muscle damage (Paulsen *et al.,* 2012; Warren *et al.,* 1999), the finding that a single WBC treatment was ineffective in alleviating soreness or plasma CK is not of major concern. The mechanisms of muscle soreness likely involve mechanical hyperalgesia and activation of muscle nociceptors (Peake *et al.,* 2017). It is reasonable to conclude that WBC is ineffective in mediating these pain sensation mechanisms, despite its potential in reducing nerve conduction velocity (Bouzigon *et al.,* 2016). Exploring in further depth the possible mechanisms by which WBC can treat muscle damage post-exercise remains a potential avenue for further research.

7.2. Inter-individual differences in responses to cryotherapy and muscle damage

The inter-individual variability in response to cryotherapy is evident (Cuttell *et al.,* 2017; Hammond *et al.,* 2014) and could partially explain discrepant findings on the efficacy of WBC for post-exercise recovery. In the current body of work there was a

significant effect of age on the post-exercise recovery response to WBC with regards to muscle strength, which partially supported the initial hypothesis (chapter 4). Despite a lack of influence of ageing on physiological variables (e.g. skin temperature), this significant finding highlights the importance of considering inter-individual variability before prescribing post-recovery interventions for sports and exercise. As explained in chapter 4, the reasons behind the lack of WBC benefit for muscle strength post-EIMD in the older group are not clear, but it is conceivable that differences in muscle mass and/or inflammation is a factor.

It has previously been shown that body fat content significantly affects the response to CWI post-exercise (Stephens *et al.,* 2018), perhaps via more pronounced reductions in core temperature. There is lack of research to investigate the role of body compositions on responses to WBC post-exercise, hence the rationale for the first main study (chapter 4). It was found that body fat content did not significantly influence post-exercise recovery following WBC, including physiological variables, rejecting the initial hypothesis. It should be noted that the study in chapter 4 assessed markers within 10 minutes after cryotherapy and then at 24 hours post, as opposed to a continual time period for more than 30 minutes, as was performed by other studies reporting significant influences of body fat percentage (Cuttell *et al.,* 2017; Stephens *et al.,* 2018).

The inter-individual variability in response to muscle damage should also be considered (Fatourous & Jamurtas, 2016). There did not appear to be any difference between age/body fat groups in damage responses to the downhill run independent of WBC. For instance, muscle torque in the CON group significantly decreased regardless of age or body fat percentage (chapter 4, data not shown). As mentioned previously (section 2.6.5), inter-individual variability in responses to EIMD could influence the effectiveness of cryotherapy interventions. Body fat percentage has recently been shown to influence the muscle damage response following isolated eccentric arm curls (Yoon & Kim, 2020), but this does not appear to be the case following a downhill run.

Another factor which can contribute to the inter-individual variability to muscle damaging exercise is fitness levels and training status (Hody *et al.,* 2019) which is a potentially important consideration due to the diversity of training backgrounds in sports participation. Despite being physically active, the participants in this body of work did not have exceptionally high aerobic capacities (mean relative VO_2 max of

47.8, 43.3 and 44.8 ml/min/kg for the three main studies) and only three participants in the entire cohort had a relative VO_2 max higher than 60 ml/min/kg. The findings of this thesis could therefore be applied to a more general active male population, rather than specifically targeting elite sport. Based on their physical activity reports on the questionnaire (Appendix 3), it was highly unlikely that they were accustomed to eccentrically biased exercises.

7.3. Optimal Treatment Protocol Factors

Understanding how different WBC protocols can ameliorate the effects of muscle damage can aid practitioners in the application of WBC strategies to facilitate recovery needs and athletic performance. Considerations of optimal WBC treatment protocols have not been comprehensively addressed in the literature, rather only superficially (Bouzgon *et al.,* 2016; Lombardi *et al.,* 2017) and experimental studies manipulating specific protocol factors are rare (Selfe *et al.,* 2014). The standard protocol for WBC is three minute exposure at a temperature range of -110°C to -140°C (Costello *et al.,* 2012; Hausswirth *et al.,* 2013; Kruger *et al.,* 2015). However further studies to clarify an optimum protocol by specifically manipulating temperature or duration may not be of great benefit for researchers considering the costs involved. A treatment temperature of -120°C was selected as is falls within the -110 to -140°C range utilised by the majority of WBC studies. As explained earlier (section 2.6.2), temperature alone is unlikely to significantly influence the physiological and recovery response to WBC post-exercise.

Combining the findings of the first two main studies, single three minute WBC treatments at -120°C taken within one hour post-exercise appears to be a favourable modality for supporting recovery. The beneficial outcome regarding muscle strength recovery (chapter 4) is consistent with other WBC studies reporting benefits when the treatment was applied between 45 and 60 minutes post-exercise (Fonda & Sarabon, 2013; Kruger *et al.,* 2015). The second main study (chapter 5) is the first to manipulate WBC treatment timing as a variable. Whilst treatments 24 hours post-exercise have previously been applied (Costello *et al.,* 2012), there is a scarcity of WBC studies that have investigated the recovery response to treatments taken between 1 and 24 hours post-exercise. The findings from the second main study reveal that WBC treatments taken 4 hours after exercise did not mitigate muscle strength reductions, with the extent of EIMD markers being very similar to the CON

group. This could be explained by the time course of muscle damage and breakdown mechanisms post-exercise (Peake *et al.,* 2017), since at several hours post-exercise, recovery interventions would be too late to cause any meaningful benefit. According to the lack of increase in plasma IL-6, the downhill run in this thesis did not appear to cause a pronounced systemic inflammatory response. Previous downhill run studies have reported significant IL-6 increases in the 1-12 hour period post-exercise (Dolci *et al.,* 2015; Fortes *et al.,* 2013; Smith *et al.,* 2009). These studies utilised downhill runs of 60 minutes duration instead of 30 minutes, which may explain the more pronounced increase of inflammatory markers. Additional measurement techniques such as muscle biopsies and immunohistochemistry (e.g. for assessing leukocyte infiltration) would provide further insight into the means in which cryotherapy can mediate the primary and secondary muscle damage mechanisms throughout the time course of recovery.

Despite the lack of influence of WBC timing on other variables, the lack of impact of WBC4 on muscle strength would indicate that single treatments taken one hour post-exercise are preferable for supporting athletic recovery. Due to cryotherapy chamber access limitations and circadian rhythm challenges, numerous treatments at between 6 and 12 hours post-exercise would have been unfeasible in this body of work.

The debate surrounding the optimum frequency of cryotherapy treatments for beneficial effects remains contentious. It is conceivable that a higher number of treatments induce more pronounced physiological benefits for exercise recovery, with a possible dose-response effect. This has been supported by several studies noting significant benefits following multiple treatments (Lubkowska *et al.,* 2011; Lubkowska *et al.,* 2012; Ziemann *et al.,* 2012); yet such studies have not reviewed the economic consequences of highly frequent WBC treatments. This thesis (chapter 4) demonstrates benefits of a single treatment which could have significant economic implications for sports practitioners. Whilst the possibility of regular WBC treatments interfering with training adaptations was considered, the training study (chapter 6) revealed no significant negative impact, especially with regards to muscle strength. Thus, single WBC treatments can be repetitively applied (i.e. on separate training days) to support post-exercise recovery without much concern on the ability to adapt to training stimuli. Since standardised trials manipulating the frequency of treatments are potentially impractical and uneconomical to conduct, researchers should focus on the potential benefits and applications of singular treatments.

7.4. Comparing Whole Body Cryotherapy with Cold Water Immersions

One of the objectives of this thesis was to compare the effectiveness of WBC with CWI for promoting post-exercise recovery. This remains a topic of interesting debate within the literature and sports science community, since benefits have been reported from both modalities, yet there are clear disparities in cost and accessibility. The findings from the second main study (chapter 5) reveal that a single WBC treatment was no more effective for post-downhill run recovery than CWI, with similar responses in muscle damage markers, thermoregulatory variables and sleep. This would add further doubt to the application of WBC treatments for sports recovery when the cheaper and more accessible alternative is just as effective. As highlighted earlier (sections 2.3.2 and 5.1.5), the body of literature comparing these two cold treatment modalities generally indicates a lack of clear benefit of WBC compared to CWI.

Whilst several of the mechanisms between WBC and CWI are similar, for instance reductions in skin, muscle and core temperatures (Costello *et al.,* 2012), reductions in cutaneous vascular conductance (Hoheanuer *et al.,* 2020) and increased parasympathetic reactivation (Hausswirth *et al.,* 2013; Ihsan *et al.,* 2016), one notable difference is the hydrostatic effect imposed by cold water, as explained previously (section 2.3.2). This might partially explain the finding that significant leg blood flow reductions were observed following CWI but not following WBC (chapter 5), as well as previous observations that CWI elicits more pronounced reductions in leg blood flow than WBC (Mawhinney *et al.,* 2017). This discrepancy in blood flow response could also favour CWI with regards to clearing away muscle metabolites and waste products via fluid shifts from the interstitial space due to possible reductions in muscle metabolism (Ihsan *et al.,* 2016). There appears to be some evidence to support this theory. In main study 2 (chapter 5), the CWI participants had the lowest peak of muscle soreness at 38%, whilst the WBC participants were closer to 50%. Although this difference was not statistically significant, other studies also indicate that CWI may be preferable for alleviating post-exercise muscle soreness (Ascensão *et al.,* 2011; Bailey *et al.,* 2007; Rossato *et al.,* 2015), which might be linked to its potential effect in reducing swelling and odema (White & Wells, 2013).

Despite the lack of benefits of WBC over CWI, there remains the possibility that WBC could actually be superior for short term (i.e. same day) recovery. This was not investigated in this thesis, since the recovery at 24 hours post-exercise was

considered more relevant, as explained in section 4.4. Nonetheless, several athletes may still be required to perform multiple training sessions and/or competitions within the same day, so the possibility of WBC offering superior benefits in this shorter time frame should not be ignored. More severe reductions in leg blood flow from CWI may mean athletes have to wait longer to participate effectively in further sessions. Some earlier findings may support this theory. For instance, better vertical jump performances were noted following partial body cryotherapy (PBC) compared to CWI 60 minutes after a drop jump protocol (Hohenauer *et al.,* 2017; Hohenauer *et al.,* 2020). Ferreira-Junior *et al.* (2014) also report short term (within one hour) benefits to muscle strength recovery following PBC. Studies on short term recovery following CWI are scarce, however some research indicates a potential negative effect (Garcia-Manso *et al.,* 2011). The theory behind this is that CWI-induced reductions in muscle temperature leads to delays in action potentials, thus force generating capacity (Machado *et al.,* 2016). However, such mechanisms have not been proposed following WBC and due to the established differences in limb blood flow, it is conceivable that WBC would be beneficial for short term exercise recovery compared to CWI. It would therefore be appropriate for future comparative studies to explore this possibility in greater depth.

In the context of training programming, the finding that repetitive WBC was not detrimental to adaptations (particularly for muscle strength) may lend further support to the use of WBC instead of CWI. As highlighted in chapter 6, the few previous studies investigating the impact of repetitive WBC on training adaptations also did not report negative effects (Broatch *et al.,* 2019; Jaworska *et al.,* 2018). This is in contrast to previous CWI studies (Roberts *et al.,* 2015; Yamane *et al.,* 2006). Therefore, repeated CWI may cause a larger hindrance effect than WBC. A possible explanation to support this theory is the more pronounced decrease in limb blood flow and consequently amino acid supply post-CWI. If muscle blood flow is not reduced to the same extent following WBC compared to CWI, then repetitive application may not interfere with the muscular adaptive response to training. Combining the findings of these earlier studies and the training study in this thesis, it is conceivable that WBC application is favourable for repetitive use in training programming. Of course, further investigation is necessary to clarify this further, whilst considering the potential negative influence of repetitive WBC on explosive power development.

7.5. Potential Applications for Sports Practice

The findings from this body of work could be used by the sporting community in a variety of ways. Whilst downhill running is not a common exercise mode utilised in sports, the nature of the exercise as well as the muscle damage response would be of relevance to several athletes aiming to enhance recovery after strenuous exercise. As explained previously (section 4.1) the whole body and multi-joint nature of downhill running makes this exercise protocol more sports specific than other commonly used models such as drop jumps and isolated arm curls. Several team sports such as association football and rugby would also observe substantial muscle damage markers comparable to those in this thesis. Such sports entail numerous eccentric muscle contractions in a variety of planes and explosive muscle damaging movements, which lead to elevated muscle inflammation and blood CK (Ascensao *et al.,* 2011; Twist & Highton, 2013). Additionally, it could be argued that the relatively high cardiorespiratory demands of such sports (Bangsbo *et al*., 2006) supports the use of downhill running as an exercise modality to investigate sports recovery interventions. Since it has been demonstrated that single WBC treatments taken one hour post-exercise can alleviate muscle damage, particularly for young males, several athletes could undergo treatments to support subsequent muscle strength and performance. As explained earlier (section 4.4), caution should be exerted in concluding the beneficial impact of WBC, for instance there was a lack of impact of WBC on muscle soreness or CK levels.

The training study (chapter 6) outcomes should be of interest to several sports practitioners. The main finding that repetitive WBC did not blunt strength adaptations would have positive implications for sports periodization and training programming. However, the potential negative impact on muscle power development is subject to interpretation and further review. It may consequently be preferable for athletes to undergo repetitive WBC treatments throughout training phases when there is higher priority on general strength development than explosive power. For instance, strength development would be prioritised for many sports during the general adaptation phase where there would be more focus on taking advantage of the general adaptation syndrome via supercompensation (Turner, 2011). Alternatively, repetitive WBC can be applied during competitive phases of athletic training programming, when athletes are more concerned with exercise recovery as opposed to training adaptations. The main cautionary note that should be applied to the

training study findings with regards to sports practice is that WBC was not superior to CON in any of the training adaptation variables – i.e. repetitive WBC does not *augment* adaptive responses to exercise training. The premise of using repetitive WBC would be to support recovery without negative consequences in training programmes (particularly involving several eccentric contractions), rather than augmenting adaptations.

7.6. Recommendations for Future Research

Further studies should focus on the effects of single WBC treatments post-exercise in more depth by examining its effect on muscle damage markers in conjunction with physiological parameters. For instance, as explained in section 5.4, measuring leg blood flow at more time points post-exercise would provide further insight into the recovery response to exercise and cold treatments. Additionally, it would be beneficial to further investigate the potential influence of cryotherapy interventions on the inflammatory response (assessed by a variety of inflammatory markers) during the first few hours after exercise. Potentially useful physiological markers that were not measured in this thesis, including muscle tissue oxygenation and electromyography (EMG), could be applied in the short term recovery period. Measuring such short term responses post-WBC would also provide more insight into how the physiological mechanisms of WBC (e.g. skin temperature reductions and subsequent cold-induced vasodilation) could mediate potential improvements in post-exercise recovery. Regarding its potential merit for same day recovery, further experimental trials directly comparing WBC with CWI should be performed assessing the aforementioned variables in order to justify the use of the more extreme, yet more expensive cold modality.

The mechanisms of ageing in response to WBC post-exercise requires further investigation following the finding that older participants did not retain their muscle torque as well as their younger counterparts. Despite the established effects of ageing with regards to exercise capacity (McCormick & Vasilaki, 2018) and cold exposure (Young *et al.,* 1996), the precise implications for post-exercise cryotherapy interventions is subject to further evaluation.

Regarding its potential repetitive use in training programmes, further studies should attempt to clarify the mechanisms of power development as well as the possibility of

repetitive WBC intervening with such adaptations. This remains an area of uncertainty following the findings of this thesis due to the lack of interference of repetitive WBC on muscular strength. It would be beneficial for future research to measure parameters such as EMG, stretch-shortening cycle capacities as well as muscle hypertrophy markers (e.g. protein synthesis rates and muscle fibre cross sectional areas) in order to elucidate how repetitive WBC could mediate potential mechanisms of strength and power development. Since power is an important consideration for several sports (Young, 2006), further clarification could be of notable significance for sports practitioners concerned with optimum results from training programmes.

Finally, due to morphological and hormonal variations, it was considered appropriate to only examine male participants in this body of work. Nonetheless, the impact of WBC in females is a research area that is in its infancy with only a handful of recent investigations (Cuttell *et al.,* 2017; Hohenauer *et al.,* 2020). Since the participation of females in sport continues to rise considerably (Senne, 2016), the potential influence of cryotherapy as a strategy for post-exercise recovery in females warrants further review.

7.7. Final Conclusions and Summary of Objectives

In relation to the objectives of this thesis as specified in chapter 1, the overall conclusions can be summarised as follows:

1) A single WBC treatment alleviates the muscle strength decrement following a muscle damaging downhill run, thereby supporting its use for post-exercise recovery. However, WBC is ineffective in treating muscle soreness or mitigating blood markers of muscle damage.
2) Young males respond better to WBC treatments post-EIMD than older males with regards to muscle strength recovery, although there was no influence of ageing on other variables. Body fat percentage generally has little impact on the response to WBC post-exercise.
3) Treatment timing does not have a significant influence on the response to WBC for post-exercise recovery. However, taking treatments within one hour after exercise is likely to be more beneficial for muscle strength recovery due to delayed WBC treatments not mitigating muscle strength decrements.
4) WBC is no more effective for treating EIMD than CWI. Therefore, the cost vs. reward implications of WBC treatments would need further reviewing (chapter 8 for economics review).
5) Repetitive WBC generally does not have a negative impact on training adaptations, especially with regards to muscle strength and potential neural adaptations and muscle hypertrophy; however, it might hinder improvements in jump power.

CHAPTER 8 -
ECONOMICS REVIEW
OF WHOLE BODY CRYOTHERAPY CHAMBER
AT CHRIS MOODY CENTRE

This review outlines the different categories by which usage of the cryogenic chamber at the Chris Moody Rehabilitation Centre (CMC) in Moulton might have impacted the costs of the research. Despite the established effects of whole body cryotherapy (WBC) and its emerging use as a method of post-exercise recovery, the commercial implications of running a WBC chamber to benefit sports practice needs to be considered. Each category is broken down and explained in more detail.

8.1. Installation costs

One of the reasons why WBC units are scarce in the UK is the considerable cost of **installation**. The CMC chamber cost over **£112,000** to install, making it questionable whether it is an effective investment when athletes and patients have easy access to alternative forms of cryotherapy such as ice packs and baths. The chambers being referred to here are the 'two stage' kinds where a vestibule chamber for familiarisation precedes the chamber proper for the main cold treatment – typically below -100°C. Whilst the initial installation expense is significant, there are also depreciation costs to take into account. A significant portion of the installation costs would consist of the heat exchanger system which requires liquid nitrogen for effective operation and is responsible for temperature control within the chambers. The heat exchanger system consists of three components – cooling, air preparation and control – all of which requires routine maintenance. Whilst the installation cost is not a factor to affect the research in this body of work directly, it is an important consideration for determining the overall efficacy and value of WBC treatment compared to other forms.

8.2. Annual costs and categories

1. Chemicals

Nitrogen and oxygen are the two main chemicals that the cryogenic chamber uses and constitute the largest expense for WBC treatments following installation. The 2015/16 academic year (the first full academic year during which this research was undertaken) observed an increase in the expenditure of the CMC attributed to **chemicals** compared to 2014/15. This expenditure accounted to **£30,923**, which was also 72% of the overall yearly confirmed expense for WBC use. However, in following years, the overall chemical expenditure reduced, such that the 2018/19 academic year cost **£20,647** (Table 8.1), which accounted to 61% of the total confirmed chamber expenditure that

year. This was likely due to reduced demand with clients in the final years, particularly as the CMC and the WBC chamber was commercially discontinued at the end of 2019. The significant chemical expense covers the cooling process of the cryogenic chamber from room temperature to the desired temperature of -120°C and typically lasts up to 30 minutes. To make the experiments more economical, it was beneficial to apply the treatment to multiple participants (e.g. training study, chapter 6) on occasions whilst the temperature was at the required value. The whole body unit could hold up to four participants in one treatment and there was theoretically no limit on how long the chamber could be maintained at the desired temperature.

2. Cryogenic Storage Vessels

The second most significant annual expenditure was the **rental of cryogenic storage vessels.** BOC were the CMC's suppliers of the storage vessels. This accounted to **£9610** for the 2015/16 academic year and **£10,618** in 2018/19 (22% and 31% of total confirmed annual costs respectively).

3. Maintenance

The **maintenance** costs are also potentially significant. Typically, this would account for an expenditure of around **£1500** per annum, depending on the amount of repair required. In the 2015/16 academic year, no service costs were required, although exact figures for subsequent years are not available. The heat exchanger system needed to be routinely maintained, as well as the air purification system via air filters and compressors. Additionally, the lining of the chamber walls needed to be checked on a regular basis to minimise harmful effects of skin contact with low temperatures, as well as the chamber drying system (e.g. valves, circulating fan) and oxygen concentration control valves. Unfortunately, the CMC were unable to provide a record of the exact maintenance activities that was performed throughout this research.

4. Sundries

Variable costs for miscellaneous items (e.g. masks, head bands, gloves etc.) and replacements would need to be accounted for. The expense for general **sundries** totalled **£2236.35** in 2015/16 (approximately 5% of the confirmed WBC expense) and **£2789** in 2018/19 (8%).

5. Labour

It is clearly necessary to employ staff to run and operate cryogenic chambers, as well as administer bookings. The cost of **labour** for the CMC in 2016 was **£8.75 per hour** for part time staff and **£10.71 per hour** for full time staff. Each cryotherapy session would likely require approximately 30 minutes of labour time, considering the time required for starting up the chamber, completing medical forms, subject supervision and deactivating/drying. Assuming a 50:50 ratio of full time and part time CMC staff (**average £9.73 per hour**), each session would cost an estimated **£4.87 for labour**. Since there were 206 WBC sessions in 2015/16, the accumulated labour expenditure associated with WBC usage would have been **£1003** (see Table 8.1).

These costs were unlikely to have a direct impact on my research since the chamber was used at times when staff was already present. Additionally, several of the WBC treatments in this body of work (particularly in later years and for the training study in chapter 6) were self-operated without CMC staff assistance. Therefore, it is difficult to quantify the exact costs associated with labour.

6. Electricity

Another factor that is difficult to evaluate is the routine expenditure associated with electricity. The cryochamber installed at the CMC had a power output of 3.5kW. On average, single WBC sessions consumed power for approximately an hour, considering the time required for cooling to the required temperature and drying. According to the Department for Business, Energy & Industrial Strategy, the average cost of electricity for a medium non-domestic consumer is 14p per kWh. Thus, each WBC session in the CMC would be estimated to cost **£0.49** (3.5kWh x £0.14). There were 206 sessions of WBC throughout the 2015/16 academic year (before data collection for this thesis commenced), therefore the estimated expenditure attributed to electricity was **£100.94**. However, this does not take into account other electricity usage that would be necessary for cryotherapy treatments (e.g. lighting).

The actual expenses directly attributed to the CMC cryotherapy chamber are summarised in Table 8.1. Forecasted labour and electricity expenses as well as estimated maintenance are included below to calculate the overall estimated expenditures.

Table 8.1: Summary of expenses for WBC chamber per academic year. * Labour forecast based on £4.87 per session. ** Electricity forecast based on £0.49 per session. The annual usages of the WBC chamber are in Table 8.2.

Category	2018-19	2017-18	2016-17	2015-16
Chemicals	£20,647.47	£25,332.88	£25,962.16	£30,923.23
Sundries	£2788.50	£3,789.66	£4,320.94	£2,236.35
Storage vessels	£10613.88	£8,233.44	£10,852.38	£9,609.96
TOTAL CONFIRMED EXPENDITURE	**£34,050**	**£37,356**	**£41,135**	**£42,770**
Extra expense forecast – labour *	£730.5	£905.82	£715.89	£1003.22
Extra expense forecast – electricity **	£73.5	£91.14	£72.03	£100.94
Estimated maintenance	£1500	£1500	£1500	£0
TOTAL ESTIMATED EXPENDITURE	**£36,354**	**£39,853**	**£43,423**	**£43,874**

8.3. Costs per cryotherapy session

The projected costs of single WBC sessions for 2015-2019 are presented in Table 8.2.

Table 8.2: Breakdown of costs based on single WBC sessions. * number of treatments include those used for this research (in brackets) as well as externally (clients, sports clubs etc.)

	2018-19	2017-18	2016-17	2015-16
Number of cryotherapy sessions*	150 (56)	186 (13)	147 (6)	206
Total Estimated Expenditure	**£36,354**	**£39,853**	**£43,423**	**£43,874**
Average cost per session	**£242.36**	**£214.26**	**£295.40**	**£212.90**

Average estimated cost per WBC session from years 2015-2019 = **£240.86**

(£235.99 excluding labour)

The Chris Moody Centre charged clients £55 for single treatments of WBC, which was not applied to the participants in this research. The estimated CMC shortfalls for each academic year were thereby calculated as follows:

2015/16: 206 WBC treatments x £55 = £11,330 income
=> Total shortfall of £43,874 - £11,330 = £32,544 = **£158 shortfall per session**

2016/17: 141 WBC treatments x £55 = £7755 income
=> Total shortfall of £43,423 - £7755 = £35,668 = **£243 shortfall per session**

2017/18: 173 WBC treatments x £55 = £9515 income
=> Total shortfall of £39,853 - £9515 = £30,338 = **£163 shortfall per session**

2018/19: 94 WBC treatments x £55 = £5170 income
=> Total shortfall of £36,354 - £5170 = £31,184 **= £208 shortfall per session**

Average shortfall for years 2015-19 = **£193 per session**

8.4. Research specific costs

The total cryotherapy usage throughout this body of work was 152 treatments, the majority (77) of which occurred between September and December 2019 before the CMC was dissolved, hence these sessions are not accounted for in Tables 8.1 and 8.2. The 152 sessions includes all WBC treatments for pilot study 2 (section 3.5) and the three main studies (chapters 4-6). It also takes into account numerous times where participants underwent treatments simultaneously, some extra sessions that did not directly contribute to the data collection (e.g. marketing purposes, BBC Radio Northampton) and trial runs. Based on 152 treatments, the associated costs (excluding labour since many treatments were self-operated) is calculated as follows:

£235.99 per WBC treatment x 152 = **£35,870** throughout research.

8.5. Final economic analysis perspectives

Such figures and calculations are based on the cryogenic chamber at the Chris Moody Centre at Moulton and it is likely that the precise economic implications of WBC treatments elsewhere would be different. Certainly, the total expenditure of the WBC chamber is significant and should be taken into account before applying the treatment to benefit sports practice. Therefore, it is necessary to consider ways to economise the treatment whilst still maintaining benefits for athletic recovery and performance. The finding that a single WBC treatment benefited muscle strength following muscle damage (chapter 4) is noteworthy since it would highlight that multiple treatments may not be necessary to experience substantial benefits. However, the subsequent finding that WBC was no more effective than cold water immersion (chapter 5) means that the overall cost-reward implications of WBC remains contentious. Since only two weekly WBC treatments did not appear to hinder training adaptations (chapter 6), sports and exercise practitioners should still be able to cautiously apply repetitive treatments in an economical manner to benefit recovery and performance.

References

Abaïdia, A. E., Lamblin, J., Delecroix, B., Leduc, C., McCall, A., Nédélec, M., Dawson, B., Baquet, G., & Dupont, G. 2017. Recovery from exercise-induced muscle damage: cold-water immersion versus whole-body cryotherapy. *International Journal of Sports Physiology and Performance*. 12(3): 402-409

Achten, J., & Jeukendrup, A. E. 2003. Heart rate monitoring: Applications and limitations. *Sports Medicine*. 33(7): 517-538.

Al Haddad, H., Parouty, J., Buchheit, M. 2012. Effect of daily cold water immersion on heart rate variability and subjective ratings of well-being in highly trained swimmers. *International Journal of Sports Physiology and Performance.* 7(1): 33-38.

Algafly, A. A., & George, K. P. 2007. The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. *British Journal of Sports Medicine.* 41(6): 365-369.

de Araujo Ribeiro Alvares, J. B., Rodrigues, R., de Azevedo Franke, R., da Silva, B., Pinto, R. S., Vaz, M. A., & Baroni, B. M. 2014. Inter-machine reliability of the Biodex and Cybex isokinetic dynamometers for knee flexor/extensor isometric, concentric and eccentric tests. *Physical Therapy in Sport.* 16(1): 59-65.

Anton, M. M., Cortez-Cooper, M. Y., DeVan, A. E., Neidre, D. B., Cook, J. N, & Tanaka, H. 2006. Resistance training increases basal limb blood flow and vascular conductance in aging humans. *Journal of Applied Physiology.* 101(5): 1351-1355.

Argus, C. K., Gill, N. D., Keogh, J..L., McGuigan, M. R., & Hopkins, W. G. 2012. Effects of two contrast training programs on jump performance in rugby union players during a competition phase. *International Journal of Sports Physiology and Performance*. 7(1): 68-75.

Ascensão, A., Leite, M., Rebelo, A. N., Magalhäes, S., & Magalhäes, J. 2011. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. *Journal of Sports Sciences.* 29(11): 217-225.

Assumpção, C. O., Lima, L. C. R., Oliveira, F. R. D.,Greco, C. C., & Sérgio Denadai, B. 2013. Exercise-induced muscle damage and running economy in humans. *Scientific World Journal.* 189149.

Bailey, M., Erith, S.J., Griffin, P.J. Dowson, A., Brewer, D. S., Gant, N., & Williams, C. 2007. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *Journal of Sports Sciences.* 25(11): 1163 – 1170.

Baird, M. F., Graham, S. M., Baker, J. S., & Bickerstaff, G. F. 2012. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *Journal of Nutrition and Metabolism.* 2012: 960363.

Banfi, G., Melegati, G., Barassi, A., Dogliotti, G., Melzi d'Eril, G., Dugué, B., Corsi, M. M. 2009. Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. *Journal of Thermal Biology.* 34(2): 55-59.

Banfi, G., Lombardi, G., Colombini, A., & Melegati, G. 2009. Whole-body cryotherapy in athletes. *Sports Medicine.* 40(6): 509-517.

Bangsbo, J., Mohr, M., & Krustrup, P. 2006. Physical and metabolic demands of training and match-play in the elite football player. *Journal of Sports Sciences*. 24(7): 665-674.

Barnett, A. 2006. Using recovery modalities between training sessions in elite athletes: does it help? *Sports Medicine*. 36(9): 781-796.

Baross, A. W., Wiles, J. D., & Swaine, I. L. 2013. Double-leg isometric exercise training in older men. *Open Access Journal of Sports Medicine.* 4: 33–40.

Baumann, C. W., Green, M. S., Doyle, J. A., Rupp, J. C., Ingalls, C. P., & Corona, B. T. 2014. Muscle torque loss and muscle damage after downhill running. *Journal of Strength and Conditioning Research*. 28(5): 1212-1218.

Benito, P.J., Cupeiro, R., Ramos-Campo, D. J., Alcaraz, P. E., & Rubio-Arias, J. Á. 2020. A Systematic Review with meta-analysis of the effect of resistance training on whole-body muscle growth in healthy adult males*. International Journal of Environmental Research and Public Health.* 17(4): 1285.

Bettoni, L., Bonomi, F. G., Zani, V., Manisco, L., Indelicato, A., Lanteri, P., Banfi, G., & Lombardi, G. 2013. Effects of 15 consecutive cryotherapy sessions on the clinical output of fibromyalgic patients. *Clinical Rheumatology. 3*2(9): 1337-1345.

Bieuzen, F., Hausswirth, C., & Dugué B. 2019. Circulating soluble intercellular adhesion molecule-1 (sICAM-1) after exercise-induced muscular damage: Does the use of whole-body cryostimulation influence its concentration in blood? *Cryobiology*. 87: 120-122.

Bilsborough, J. C., Greenway, K., Opar, D., Livingstone, S., Cordy, J., & Coutts, A. J. 2014. The accuracy and precision of DXA for assessing body composition in team sport athletes. *Journal of Sports Sciences.* 32(19): 1821-1828.

Blanco, P. 2015. Volumetric blood flow measurement using Doppler ultrasound: concerns about the technique. *Journal of Ultrasound*. 18(2): 201-204.

Bleakley, C. M., & Davison, G. W. 2010. Cryotherapy and inflammation: evidence beyond the cardinal signs. *Physical Therapy Reviews*. 15(6): 430-435.

Bleakley, C. M., & Hopkins, J. T. 2010. Is it possible to achieve optimal levels of tissue cooling in cryotherapy? *Physical Therapy Reviews*. 15(4): 344-350.

Bleakley, C. M., Bieuzen, F., Davison, G. W., Costello, J. T. 2014. Whole-body cryotherapy: empirical evidence and theoretical perspectives. *Open Access Journal of Sports Medicine.* 10(5): 25-36.

Bouzigon, R., Ravier, Gilles., Dugué, B., & Grappe, F. 2014. The use of whole-body cryostimulation to improve the quality of sleep in athletes during high level standard competitions. *British Journal of Sports Medicine.* 48: 572.

Bouzigon, R., Grappe, F., Ravier, G., & Dugue B. 2016. Whole- and partial-body cryostimulation/cryotherapy: Current technologies and practical applications. *Journal of Thermal Biology.* 61: 67-81.

Brancaccio, P., Maffulli, N. & Limongelli, F. M. 2007. Creatine kinase monitoring in sport medicine. *British Medical Bulletin*. 81-82, 209-230.

Broatch, J. R., Petersen, A., & Bishop, D. J. 2014. Post-exercise cold water immersion benefits are not greater than the placebo effect. *Medicine and Science in Sports and Exercise.* 46(11): 2139–2147.

Broatch, J. R., Petersen, A., & Bishop, D. J. 2017. Cold-water immersion following sprint interval training does not alter endurance signalling pathways or training adaptations in human skeletal muscle. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*. 1;313(4): R372-R38

Broatch, J.R., Poignard, M., Hausswirth, C., Bishop, D. J. & Bieuzen, F. 2019. Whole-body cryotherapy does not augment adaptations to high-intensity interval training. *Scientific Reports*. 9, 12013.

Brook, M. S., Wilkinson, D. J., Mitchell, W. K., Lund, J. N, Szewczyk, N. J., Greenhaff, P. L., Smith, K., & Atherton, P. J. 2015. Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. *FASEB Journal.* 29(11): 4485-4496.

Bruce, R. A., Blackmon, J. R., Jones, J. W., & Strait, G. 1963. Exercising testing in adult normal subjects and cardiac patients. *Pediatrics.* 32(4): 742-756.

.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T. L., MacLean, D. A., & Pedersen, B. K. 1997. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *Journal of Physiology.* 499(Pt 3): 833–841.

Byrnes, W. C., Clarkson, P. M., White, J. S., Hsieh, S. S., Frykman, P. N, & Maughan, R. J. 1985. Delayed onset muscle soreness following repeated bouts of downhill running. *Journal of Applied Physiology*. 59(3): 710-715.

Cannon, B., & Nedergaard, J. 2012. Yes, even human brown fat is on fire! *The Journal of Clinical Investigation*. 122(2): 486–489.

Chen, M. J. Fan, X., & Moe, S. T. 2002. Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. *Journal of Sports Sciences*. 20(11): 873-899.

Chen, T. C., Nosaka, K., & Tu, J. H. 2007. Changes in running economy following downhill running. *Journal of Sports Sciences.* 25(1): 55-63.

Chen, T. C., Nosaka, K., Lin, M. J., Chen, H. L., & Wu, C. J. 2009. Changes in running economy at different intensities following downhill running. *Journal of Sports Sciences*. 27(11): 1137-1144.

Cheung, K., Hume, P., & Maxwell, L. 2003. Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Medicine.* 33: 145–164.

Childs, C., Harrison, R., & Hodkinson, C. 1999. Tympanic membrane temperature as a measure of core temperature. *Archives of Disease in Childhood.* 80(3): 262-266.

Clark, D. R., Lambert, M. I., & Hunter, A. M. 2012. Muscle activation in the loaded free barbell squat: a brief review. *Journal of Strength and Conditioning Research.* 26(4): 1169-1178.

Clarkson, P. M., & Hubal, M. J. 2002. Exercise-induced muscle damage in humans. *American Journal of Physical Medicine & Rehabilitation.* 81(Suppl):S52–S69.

Close, G. L., Ashton, T., Cable, T. 2004. Eccentric exercise, isokinetic muscle torque and delayed onset muscle soreness: the role of reactive oxygen species. *European Journal of Applied Physiology.* 91: 615–621.

Coffey, V. G., & Hawley, J. A. 2017. Concurrent exercise training: do opposites distract? *Journal of Physiology.* 595(9): 2883-2896.

Collier, S. R., Kanaley, J. A., Carhart, R., Frechette, V., Tobin, M. M., Hall, A. K., Luckenbaugh, A. N., & Fernhall, B. 2008. Effect of 4 weeks of aerobic or resistance exercise training on arterial stiffness, blood flow and blood pressure in pre- and stage-1 hypertensives. *Journal of Human Hypertension.* 22(10): 678-686.

Collins, S. M., Silberlicht, M., Perzinski, C., Smith, S. P., & Davidson, P. W. 2014. The relationship between body composition and preseason performance tests of collegiate male lacrosse players. *Journal of Strength and Conditioning Research.* 28(9): 2673-2679.

Costello, J. T., McInerney, C. D., Bleakley, C. M., Selfe, J., & Donnelly, A. E. 2012. The use of thermal imaging in assessing skin temperature following cryotherapy: A review. *Journal of Thermal Biology.* 37(2): 103–110.

Costello, J. T., Culligan, K., Selfe, S., & Donnelly, A. E. 2012. Muscle, skin and core temperature after -110°C cold air and 8°C water treatment. *Plos One.* 7(11). e48190.

Costello, J. T., Algar, L. A., & Donnelly, A. E. 2012. Effects of whole-body cryotherapy (-110 °C) on proprioception and indices of muscle damage. *Scandinavian Journal of Medicine and Science in Sports.* 22(2): 190-198.

Costello, J. T., Stewart, I., B., Selfe, J., Kärki, A., & Donnelly, A. E. 2013. Use of thermal imaging in sports medicine research: A short report. *International SportMed Journal*. 14(2): 94-98.

Costello, J. T., Donnelly, A. E., Karki, A., & Selfe, J. 2014. Effects of whole body cryotherapy and cold water immersion on knee skin temperature. *International Journal of Sports Medicine*. 35(1): 35-40.

Costello, J. T., Baker, P. R., Minett, G. M., Bieuzen, F., Stewart, I. B., & Bleakley, C. 2015. Cochrane review: Whole-body cryotherapy (extreme cold air exposure) for preventing and treating muscle soreness after exercise in adults. *Cochrane Database of Systematic Reviews.* 18(9). CD010789.

Crouter, S. E, Albright, C., & Bassett, D. R. 2004. Accuracy of polar S410 heart rate monitor to estimate energy cost of exercise. *Medicine and Science in Sports and Exercise.* 36(8): 1433-1439.

Crystal, N. J., Townson, D. H., Cook, S. B., & LaRoche, D. P. 2013. Effect of cryotherapy on muscle recovery and inflammation following a bout of damaging exercise. *European Journal of Applied Physiology.* 113: 2577–2586.

Cuttell, S., Hammond, L., Langdon, D., & Costello, J. 2017. Individualising the exposure of −110 °C whole body cryotherapy: The effects of sex and body composition. *Journal of Thermal Biology.* 65: 41-47.

Del Vecchio, A., Casolo, A;, Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., & Farina, D. 2019. The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *Journal of Physiology.* 597(7): 1873-1887.

Dolci, A., Fortes, M. B., Walker, F. S., Haq, A., Riddle, T., Walsh N. P. 2015. Repeated muscle damage blunts the increase in heat strain during subsequent exercise heat stress. *European Journal of Applied Physiology*. 115(7): 1577-1588.

Douzi, W., Dupuy, O., Tanneau, M., Boucard, G., Bouzigon, R., & Dugué, B. 2018. 3-min whole body cryotherapy/cryostimulation after training in the evening improves sleep quality in physically active men. *European Journal of Sport Science*. 19(6): 860-867.

Douzi, W., Dupuy, O., Theurot, D., Boucard, G., & Dugué, B. 2019. Partial-body cryostimulation after training improves sleep quality in professional soccer players. *BMC Research Notes.* 12(1): 141.

Earp, J. E., Hatfield, D. L., Sherman, A., Lee, E. C., & Kraemer, W. J. 2019. Cold-water immersion blunts and delays increases in circulating testosterone and cytokines post-resistance exercise. *European Journal of Applied Physiology.* 119(8): 1901-1907.

Esco, M. R., Fedewa, M. V., Cicone, Z. S., Sinelnikov, O. A., Sekulic, D., Holmes, C. J. 2018. Field-Based performance tests are related to body fat percentage and fat-free mass, but not body mass index, in youth soccer players. *Sports.* 6(4): 105.

Evenson, K. R., Goto, M. M., & Furberg, R. D. 2015. Systematic review of the validity and reliability of consumer-wearable activity trackers*. International Journal of Behavioural Nutrition and Physical Activity*. 12(159).

Fatourous, I. G., & Jamurtas, A, Z. 2016. Insights into the molecular etiology of exercise-induced inflammation. *Journal of Inflammation Research.* 9: 175-186.

Feehan, L., Geldman, J., Sayre, E. C., Park, C., Ezzat, A. M., Ju, Y. Y., Hamilton, C. B., & Li, L. C. 2018. Accuracy of fitbit devices: systematic review and narrative syntheses of quantitative data. *JMIR Mhealth and Uhealth*. 6(8): e10527.

Ferreira-Junior, J. B., Bottaro, M., Vieira, C. A., Soares, S. R., Vieira, A., Cleto, V. A., Cadore, E. L., Coelho, D. B., Simoes, H. G., & Brown, L. E. 2014. Effects of partial-body cryotherapy (- 110°C) on muscle recovery between high-intensity exercise bouts*. International Journal of Sports Medicine*. 35 (14): 1155-1160.

Ferreira-Junior, J. B., Bottaro, M., Loenneke, J. P., Vieira, A., Vieira, C. A., & Bemben, M. G. 2014. Could whole-body cryotherapy (below -100°C) improve muscle recovery from muscle damage? *Frontiers in Physiology.* 5:247.

Ferreira-Junior, J. B., Bottaro, M., Vieira, A., Siqueira, A. F., Vieira, C. A., Durigan, J. L., Cadore, E. L., Coelho, L. G., Simoes, H. G., & Bemben, M. G. 2015. One session of partial-body cryotherapy (-110 °C) improves muscle damage recovery. *Scandinavian Journal of Medicine and Science in Sports.* 524–530.

Fonda, B., & Sarabon, N. 2013. Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: A crossover study. *Scandinavian Journal of Medicine and Science in Sports.* 23: e270–e278

Fonda, B., de Nardi, M., & Sarabon, N. 2014. Effects of whole-body cryotherapy duration on thermal and cardio-vascular response. *Journal of Thermal Biology.* 42, 52-55.

Fortes, M. B., Di Felice, U., Dolci, A., & Walsh, N. P. 2013. Muscle-damaging exercise increases heat strain during subsequent exercise heat stress. *Medicine and Science in Sports and Exercise.* 45(10): 1915-1924.

Fu, Q., Zhu, J., & Van Eyk, J. E. 2010. Comparison of multiplex immunoassay platforms. *Clinical Chemistry.* 56(2): 314-318.

Fuchs, C. J, Kouw, I. W. K., Churchward-Venne, T. A., Smeets, J. S. J, Senden, J. M., Lichtenbelt, W. D. V. M, Verdijk, L. B., & van Loon L. J .C. 2020. Post-exercise cooling impairs muscle protein synthesis rates in recreational athletes. *Journal of Physiology*. 598(4): 755-772.

Gamble, P. 2006. Periodization of Training for Team Sports Athletes. *Strength and Conditioning Journal.* 28(5): 56–66.

García-Manso, J. M., Rodríguez-Matoso, D., Rodríguez-Ruiz, D., Sarmiento, S., de Saa, Y., & Calderón J. 2011. Effect of cold-water immersion on skeletal muscle contractile properties in soccer players. *American Journal of Physical Medicine and Rehabilitation*. 90(5): 356-63.

Glasgow, P. D., Ferris, R., Bleakley, C. M. 2014. Cold water immersion in the management of delayed-onset muscle soreness: is dose important? A randomised controlled trial. *Physical Therapy in Sport.* 15(4): 228-233.

Goodall, S., & Howatson, G. 2008. The effects of multiple cold water immersions on indices of muscle damage. *Journal of Sports Science and Medicine.* 7: 235-241.

Gould, D., Kelly, D., Goldstone, L., Gammon, J. 2001. Examining the validity of pressure ulcer risk assessment scales: developing and using illustrated patient simulations to collect the data. *Journal of Clinical Nursing.* 10(5): 697-706.

Grainger, A., Comfort, P., & Heffernan, S. 2019. No effect of partial-body cryotherapy on restoration of countermovement-jump or well-being performance in elite rugby union players during the competitive phase of the season. *International Journal of Sports Physiology and Performance.* 29: 1-23.

Grasso, D., Lanteri, P., Di Bernardo, C., Mauri, C., Porcelli, S., Colombini, A., Zani, V., Bonomi, F. G, Melegati, G., Banfi, G., & Lombardi, G. 2014. Salivary steroid hormone response to whole-body cryotherapy in elite rugby players. *Journal of Biological Regulators and Homeostatic Agents.* 28(2). 291-300.

Green, D. J., Spence, A., Rowley, N., Thijssen, D. H., & Naylor, L. H. 2012. Vascular adaptation in athletes: is there an 'athlete's artery'? *Experimental Physiology.* 97(3): 295-304.

Gregson, W., Black, M. A., Jones, H. A., Milson, J., Morton, J., Dawson, B., Atkinson, G., & Green, D. J. 2011. Influence of cold water immersion on limb and cutaneous blood flow at rest. *American Journal of Sports Medicine.* 39(6): 1316-1323.

Gualdi-Russo, E., Toselli, S., & Squintani, L. 1997. Remarks on methods for estimating body composition parameters: reliability of skinfold and multiple frequency bioelectric impedance methods. *Zeitschrift für Morphologie und Anthropologie.* 81(3): 321-331.

Gulick, D. T., Kimura, I. F., Sitler, M., Paolone, A., & Kelly, J. D. 1996. Various treatment techniques on signs and symptoms of delayed onset muscle soreness. *Journal of Athletic Training.* 31(2): 145–152.

Haff, G. G., & Triplett, N. T. 2016. Essentials of strength training and conditioning. *Human Kinetics.* 4th Ed.

Halson, S. L., Bartram, J., West, N., Stephens, J., Argus, C. K., Driller, M. W., Sargent, C., Lastella, M., Hopkins, W. G., & Martin, D. T. 2014. Does hydrotherapy help or hinder adaptation to training in competitive cyclists? *Medicine and Science in Sports and Exercise.* 46(8): 1631–1639.

Halson, S. L. 2015. Recovery techniques for athletes. *Aspetar Sports Medicine Journal.* 4: 12-16.

Hammond, L. E., Cuttell, S., Nunley, P., & Meyler, J. 2014. Anthropometric characteristics and sex influence magnitude of skin cooling following exposure to whole body cryotherapy. *Biomed Research International.* 628724.

Hausswirth, C., Louis, J., Bieuzen, F., Pournot, H., Fournier, J., Filliard, J. R., & Brisswalter, J. 2011. Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners. *Plos One.* 6(12): e27749.

Hausswirth, C., Schaal, K., Le Meur Y., Bieuzen, F., Filliard, J. R., Volondat, M., & Louis, J. 2013. Parasympathetic activity and blood catecholamine responses following a single partial-body cryostimulation and a whole-body cryostimulation. *PLoS One.* 8(8): e72658.

Hawley, J. A., Lundby, C., Cotter, J. D., & Burke, L. M. 2018. Maximizing cellular adaptation to endurance exercise in skeletal muscle. *Cell Metabolism.* 1;27(5): 962-976.

Hayter, K. J., Doma, K., Schumann, M., & Deakin, G., B. 2016. The comparison of cold-water immersion and cold air therapy on maximal cycling performance and recovery markers following strength exercises. *PeerJ.* 28:4: e1841.

Hody, S., Croisier, J. L., Bury, T., Rogister, B., & Leprince, P. 2019. Eccentric muscle contractions: risks and benefits. *Frontiers in Physiology.* 3; 10:536.

Hohenauer, E., Costello, J. T., Stoop, R., Küng, U. M., Clarys, P., Deliens, T., & Clijsen, R. 2017. Cold-water or partial-body cryotherapy? Comparison of physiological responses and recovery following muscle damage. *Scandinavian Journal of Medicine and Science in Sports.* 28(3): 1252-1262.

Hohenauer, E., Costello, J. T., Deliens, T., Clarys, P., Stoop, R., & Clijsen, R. 2020. Partial-body cryotherapy (-135°C) and cold-water immersion (10°C) after muscle damage in females. *Scandinavian Journal of Medicine and Science in Sports.* 30(3): 485-495.

Horder, M., Jørgensen, P. J., Hafkenscheid, J. C., Carstensen, C. A., Bachmann, C., Bauer, K., Neuwald, C., Rosalki, S. B.,, Foo, A. Y., & Vogt, W. 1991. Creatine kinase determination: a European evaluation of the creatine kinase determination in serum, plasma and whole blood with the Reflotron system. *European Journal of Clinical Chemistry and Clinical Biochemistry.* 29(10): 691-696.

Huang, G., Wang, R., Chen, P., Huang, S. C., Donnelly, J. E., & Mehlferber, J. P. 2016. Dose-response relationship of cardiorespiratory fitness adaptation to controlled endurance training in sedentary older adults. *European Journal of Preventive Cardiology.* 23(5): 518-529.

Ihsan, M., Watson, G., Lipski, M., & Abbiss, C. R. 2013. Influence of post-exercise cooling on muscle oxygenation and blood volume changes. *Medicine and Science in Sports and Exercise.* 45(5): 876–882.

Ihsan, M., Watson, G., Choo, H. C., Lewandowski, P., Papazzo, A., Cameron-Smith, D., & Abbiss, C. R. 2014. Postexercise muscle cooling enhances gene expression of PGC-1α. *Medicine and Science in Sports and Exercise*. 46(10): 1900–1907.

Ihsan, M., Watson, G., Abbiss, C. R. 2016. What are the physiological mechanisms for post-exercise cold water immersion in the recovery from prolonged endurance and intermittent exercise? *Sports Medicine*. 46(8): 1095-1109.

Jaggernath, M., Naicker, R., Madurai, S. Brockman, M. A., Ndung'u, T., & Gelderblom, H. C. 2016. Diagnostic accuracy of the HemoCue Hb 301, STAT-Site MHgb and URIT-12 point-of-care hemoglobin meters in a central laboratory and a community based clinic in Durban, South Africa. *PLoS One.* 5;11(4): e0152184.

Jaworska, J., Micielska, K., Kozłowska, M., Wnorowski, K., Skrobecki, J., Radzimiński, L., Babińska, A., Rodziewicz, E., Lombardi, G., & Ziemann, E. 2018. A 2-week specific volleyball training supported by the whole body cryostimulation protocol induced an increase of growth factors and counteracted deterioration of physical performance. *Frontiers in Physiology.* 9: 1711.

Jaworska, J., Rodziewicz-Flis, E., Kortas, J., Kozłowska, M., Micielska, K., Babińska, A., Laskowski, R., Lombardi, G., & Ziemann, E. 2020. Short-term resistance training supported by whole-body cryostimulation induced a decrease in myostatin concentration and an increase in isokinetic muscle strength. *International Journal of Environmental Research and Public Health.* 17(15): 5496.

Jaworska, J., Laskowski, R., Ziemann, E., Zuczek, K., Lombardi, G., Antosiewicz, J., & Zurek, P. 2021. The specific judo training program combined with the whole body cryostimulation induced an increase of serum concentrations of growth factors and changes in amino acid profile in professional judokas. *Frontiers in Physiology*. 9;12: 627657.

Jones, A. M., & Carter, H. 2000. The effect of endurance training on parameters of aerobic fitness. *Sports Medicine.* 29(6): 373-386.

Kenney, W. L., Wilmore, J., & Costill, D. 2015. Physiology of sport and exercise. *Human Kinetics.* 6th Ed.

Knight, J., & Nigam, Y. 2008. The Anatomy and Physiology of Ageing. Part 1— The Cardiovascular System. *Nursing Times*. 104 (31), 26–27.

Kraemer, W. J., Duncan, N. D., & Volek, J. S. 1998. Resistance training and elite athletes: adaptations and program considerations. *The Journal of Orthopaedic and Sports Physical Therapy.* 28(2): 110-119.

Kraemer, W. J., & Looney, D. P. 2012. Underlying mechanisms and physiology of muscular power. *Strength and Conditioning Journal*. 34(6): 13–19.

Kruger, M., de Marees, M., Dittmar, K. H., Sperlich, B., & Mester, J. 2015. Whole-body cryotherapy's enhancement of acute recovery of running performance in well-trained athletes. *International Journal of Sports Physiology and Performance*. 10(5): 605-612.

Krueger, M., Costello, J. T., Achtzehn, S., Dittmar, K. H., & Mester, J. 2018. Whole-body cryotherapy (-110 °C) following high-intensity intermittent exercise does not alter hormonal, inflammatory or muscle damage biomarkers in trained males. *Cytokine.* 113: 277-284.

Leeder, J., Gissane, C., Someren, K. V., Gregson, W., & Howatson, G. 2012. Cold water immersion and recovery from strenuous exercise: a meta-analysis. *British Journal of Sports Medicine.* 46(4): 233-240.

Lima, C. D., Brown, L. E., Herat, N., Li, Y., & Behm, D. 2019. Periodized versus non-periodized stretch training on gymnasts flexibility and performance*. International Journal of Sports Medicine.* 40(12): 779-788.

Loenneke, J. P., Barnes, J. T., Wilson, J. M., Lowery, R. P., Isaacs, M. N., & Pujol, T. J. 2013. Reliability of field methods for estimating body fat. *Clinical Physiology and Functional Imaging.* 33(5): 405-408.

Lombardi, G., Ziemann, E., & Banfi, G. 2017. Whole-body cryotherapy in athletes: from therapy to stimulation. An updated review of the literature. *Frontiers in Physiology*. 8: 258.

Lubkowska, A., & Szyguła, Z. 2010. Changes in blood pressure with compensatory heart rate decrease and in the level of aerobic capacity in response to repeated whole-body cryostimulation in normotensive, young and physically active men. *International Journal of Occupational and Medicine and Environmental Health.* 23(4): 367-375.

Lubkowska, A., Szyguła, Z., Chlubek, D., & Banfi, G. 2011. The effect of prolonged whole-body cryostimulation treatment with different amounts of sessions on chosen pro- and anti-inflammatory cytokines levels in healthy men. *Scandinavian Journal of Clinical and Laboratory Investigations.* 71(5): 419-425

Lubkowska. A. 2012. Cryotherapy: Physiological considerations and applications to physical therapy. *Physical Therapy Perspectives in the 21st Century – Challenges and Possibilities. InTechOpen.* 155-176.

Lubkowska, A., Dołęgowska, B, & Szyguła Z. 2012. Whole-body cryostimulation--potential beneficial treatment for improving antioxidant capacity in healthy men--significance of the number of sessions. *PLoS One*. 7(10): e46352.

Lund, H., Søndergaard, K., Zachariassen, T., Christensen, R., Bülow, P,, Henriksen, M., Bartels, E. M., Danneskiold-Samsøe, B., & Bliddal, H. 2005. Learning effect of isokinetic measurements in healthy subjects, and reliability and comparability of Biodex and Lido dynamometers. *Clinical Physiology and Functional Imaging.* 25(2): 75-82.

Ma, S. Y., Je, H. D., Jeong, J. H., Kim, H. Y., & Kim, H. D. 2013. Effects of whole-body cryotherapy in the management of adhesive capsulitis of the shoulder. *Archive of Physical Medicine and Rehabilitation.* 94(1): 9-16.

Machado, A. F., Ferreira, P. H., Micheletti, J. K., de Almeida, A. C., Lemes, Í. R., Vanderlei, F. M., Netto, J. J., & Pastre, C. M. 2016. Can water temperature and immersion time influence the effect of cold water immersion on muscle soreness? A systematic review and meta-analysis. *Sports Medicine.* 46(4): 503-514.

Malm, C., Sjödin, B., Sjöberg, B., Lenkei, R., Renström, P., Lundberg, I., & Ekblom, B. 2004. Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. *Journal of Physiology.* 556 (Pt 3): 983–1000.

Marino, F. E. 2002. Methods, advantages, and limitations of body cooling for exercise performance. *British Journal of Sports Medicine.* 36: 89–94.

Markovic, G., Dizdar D, Jukic, I., & Cardinale, M. 2004. Reliability and factorial validity of squat and countermovement jump tests. *Journal of Strength and Conditioning Research.* 18(3): 551-555.

Marrier, B., Le Meur, Y., Leduc, C., Piscione, J., Lacome, M., Igarza, G, Hausswirth, C., Morin, J. B., & Robineau, J. 2018. Training periodization over an elite rugby sevens season: From theory to practice. *International Journal of Sports Physiology and Performance.* 18: 1-9.

Mattila, V. M., Tallroth, K., Marttinen, M., Pihlajamäki, H. 2007. Physical fitness and performance. Body composition by DEXA and its association with physical fitness in 140 conscripts. *Medicine and Science in Sports and Exercise.* 39(12): 2242-2247.

Mawhinney, C., Jones, H., Joo, C. H., Low, D. A., Green, D. J., & Gregson, W. 2013. Influence of cold-water immersion on limb and cutaneous blood flow after exercise. *Medicine and Science in Sports and Exercise*. 45(12): 2277–2285.

Mawhinney, C; Low, D. A., Jones, H; Green, D. J., Costello, J. T. & Gregson, W. 2017. Cold-water mediates greater reductions in limb blood flow than whole body cryotherapy. *Medicine and Science in Sports and Exercise.* 49(6): 1252-1260.

McClenton, L. S., Brown, L. E., Coburn, J. W., & Kersey, R. D. 2008. The effect of short-term VertiMax vs. depth jump training on vertical jump performance. *Journal of Strength and Conditioning Research.* 22(2): 321-325.

McCormick, R., & Vasilaki, A. 2018. Age-related changes in skeletal muscle: changes to lifestyle as a therapy. *Biogerontology.* 19(6): 519–536.

McGeough, M. D., Pena C. A., Mueller J. L., Pociask, D. A., Broderick, L, Hoffman H. M, Brydges, S. D. 2012. IL-6 is a marker of inflammation with no direct role in inflammasome-mediated mouse models. *Journal of Immunology.* 189(6).

Meyer, T., Georg, T., Becker, C., & Kindermann, W. 2001. Reliability of gas exchange measurements from two different spiroergometry systems. *International Journal of Sports Medicine.* 22(8): 593-597.

Mila-Kierzenkowska, C., Jurecka, A., Woźniak, A., Szpinda, M., Augustyńska, B., & Woźniak, B. 2013. The effect of submaximal exercise preceded by single whole-body cryotherapy on the markers of oxidative stress and inflammation in blood of volleyball players. *Oxidative Medicine and Cellular Longevity.* 409567.

Mila-Kierzenkowska, C., Woźniak, A., Woźniak, B., Drewa, G., Rakowski, A., Jurecka, A., & Rajewski, R. 2009. Whole-body cryostimulation in kayaker women: a study of the effect of cryogenic temperatures on oxidative stress after the exercise. *The Journal of Sports Medicine and Physical Fitness.* 49(2): 201-207.

Miller, E., Markiewicz, Ł., Saluk, J., & Majsterek, I. 2012. Effect of short-term cryostimulation on antioxidative status and its clinical applications in humans. *European Journal of Applied Physiology.* 112(5): 1645-1652.

Miyama, M., & Nosaka, K. 2004. Muscle damage and soreness following repeated bouts of consecutive drop jumps. *Advances in Exercise and Sports Physiology.* 10(3): 63-69.

Myer, G. D., Kushner, A. M., Brent, J. L., Schoenfeld, B. J., Hugentobler, J., Lloyd, R. S., Vermeil, A., Chu, D. A., Harbin, J., & McGill, S. M. 2014. The back squat: A proposed assessment of functional deficits and technical factors that limit performance. *Strength and Conditioning Journal*. 36(6): 4–27.

Myrer, J. W., Myrer, K. A., Measom, G. J., Fellingham, G. W., & Evers, S. L. 2001. Muscle temperature is affected by overlying adipose when cryotherapy is administered. *Journal of Athletic Training.* 36(1): 32–36.

Nelson, M. R., Conlee, R. K., & Parcell, A. C. 2004. Inadequate carbohydrate intake following prolonged exercise does not increase muscle soreness after 15 minutes of downhill running. *International Journal of Sports Nutrition and Exercise Metabolism.* 14(2): 171-184.

Nosaka, K., Sakamoto, K., Newton, M., Sacco, P. 2001. How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine and Science in Sports and Exercise.* 33(9): 1490-1495.

Otte, J. W., Merrick, M. A., Ingersoll, C. D., & Cordova, M. L. Subcutaneous adipose tissue thickness alters cooling time during cryotherapy. *Archives of Physical Medicine and Rehabilitation.* 83: 1501-1505.

Owens, D. J., Twist, C., Cobley, J. N., Howatson, G., & Close, G. L. 2019. Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? *European Journal of Sports Science.* 19(1): 71-85.

Partridge, E. M., Cooke, J., McKune, A., & Pyne, D. B. 2019. Whole-body cryotherapy: potential to enhance athlete preparation for competition? *Frontiers in Physiology.* 10: 1007.

Paulsen, G., Crameri, R., Benestad, H. B., Fjeld, J. G., Mørkrid, L., Hallén, J., & Raastad, T. 2010. Time course of leukocyte accumulation in human muscle after eccentric exercise. *Medicine and Science in Sports and Exercise.* 42(1): 75-85.

Paulsen, G., Mikkelsen, U. R., Raastad, T., & Peake, J. M. 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exercise Immunology Review.* 18: 42-97.

Pawik, M., Kowalska, J., & Rymaszewska, J. 2019. The effectiveness of whole-body cryotherapy and physical exercises on the psychological well-being of patients with multiple sclerosis: A comparative analysis. *Advances in Clinical and Experimental Medicine*. 28(11): 1477-1483.

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., & Coombes, J. S. 2005. Plasma cytokine changes in relation to exercise intensity and muscle damage. *European Journal of Applied Physiology.* 95(5-6): 514-521.

Peake, J., Nosaka, K. & Suzuki, K. 2005. Characterisation of inflammatory responses to eccentric exercise in humans. *Exercise Immunology Review*. 11: 64-85.

Peake, J. M., Neubauer, O., Gatta, P. A. D., & Nozaka, K. 2017. Muscle damage and inflammation during recovery from exercise. *Journal of Applied Physiology.* 122: 559-570.

Peiffer, J. J., Abbiss, C. R., Watson, G., Nosaka, K., & Laursen, P. B. 2010. Effect of a 5-min cold-water immersion recovery on exercise performance in the heat. *British Journal of Sports Medicine.* 44(6): 461-465.

Pilch, W., Wyrostek, J., Major, P., Zuziak, R., Piotrowska, A., Czerwińska-Ledwig, O., Grzybkowska, A., Zasada, M., Ziemann, E., & Żychowska, M. 2020. The effect of whole-body cryostimulation on body composition and leukocyte expression of HSPA1A, HSPB1, and CRP in obese men. *Cryobiology.* 94: 100-106.

Piras, A., Campa, F., Toselli, S., Di Michele, R., & Raffi, M. 2019. Physiological responses to partial-body cryotherapy performed during a concurrent strength and endurance session. *Applied Physiology, Nutrition and Metabolism.* 44(1): 59-65.

Pournot, H., Bieuzen, F., Louis, J., Fillard, J. R., Barbiche, E., & Hausswirth, C. 2011. Time-course of changes in inflammatory response after whole-body cryotherapy multi exposures following severe exercise. *Plos One.* 6(7): e22748.

Purssell, E., While, A., & Coomber, B. 2009. Tympanic thermometry - normal temperature and reliability. *Paediatric Nursing.* 21(6): 40-43.

Qu, C., Wu, Z., Xu, M., Qin, F., Dong, Y., & Wang Z, Zhao J. 2020. Cryotherapy models and timing-sequence recovery of exercise-induced muscle damage in middle- and long-distance runners. *Journal of Athletic Training.* (4): 329-335.

Ramanathan, N. L. 1964. A new weighting system for mean surface temperature of the human body. *Journal of Applied Physiology.* 19(3): 531-533.

Renata, S., Tomasz, D., Andrzej, K., Sławomir, T. 2011. Impact of 10 sessions of whole body cryostimulation on cutaneous microcirculation measured by laser Doppler flowmetry. *Journal of Human Kinetics.* 30: 75-83.

Roberts, L. A., Nosaka, K., Coombes, J. S., Peake, J. M. 2014. Cold water immersion enhances recovery of submaximal muscle function after resistance exercise. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology.* 15;307(8): R998-R1008.

Roberts., L. A., Raastad, T., Markworth, J. F., Figueiredo, V. C., Egner, I. M., Shield, A., Cameron-Smith, D., Coombes, J. S., & Peake, J. M. 2015. Post-exercise cold water immersion attenuates acute anabolic signalling and long-term adaptations in muscle to strength training. *Journal of Physiology.* 593(18): 4285-4301.

Robey, E., Dawson, B., Halson, S., Gregson, W., King, S., Goodman, C., & Eastwood, P. 2013. Effect of evening post-exercise cold water immersion on subsequent sleep. *Medicine and Science in Sports and Exercise.* 45(7): 1394-1402.

Rodríguez-Rosell, D., Mora-Custodio, R., Franco-Márquez, F., Yáñez-García, J. M., & González-Badillo, J. J. 2017. Traditional vs. sport-specific vertical jump tests: Reliability, validity, and relationship with the legs strength and sprint performance in adult and teen soccer and basketball players. *Journal of Strength and Conditioning Research.* 31(1): 196-206.

Romanowski, M.W., & Straburzyńska-Lupa A. 2020. Is the whole-body cryotherapy a beneficial supplement to exercise therapy for patients with ankylosing spondylitis? *Journal of Back and Musculoskeletal Rehabilitation.* 33(2): 185-192.

Rossato, M., Bezerraa, E. de Souza, D.A. de Ceselles Seixas da Silvaa, T. Avila Santanab, Malezamc, W. R., & Carpes, F. P. 2015. Effects of cryotherapy on muscle damage markers and perception of delayed onset muscle soreness after downhill running: A Pilot study. *Revista Andaluzade Medicina del Deporte*. 8(2): 49-53.

Rowsell, G. J., Coutts, A. J., Reaburn, P., & Hill-Haas, S. 2011. Effect of post-match cold-water immersion on subsequent match running performance in junior soccer players during tournament play. *Journal of Sports Sciences*. 29(1): 1-6.

Russell, M., Birch, J., Love, T., Cook, C. J., Bracken, R. M., Taylor, T., Swift, E., Cockburn, E., Finn, C., Cunningham, D., Wilson, L., & Kilduff, L. P., 2017. The effects of a single whole-body cryotherapy exposure on physiological, performance, and perceptual responses of professional academy soccer players after repeated sprint exercise. *Journal of Strength and Conditioning Research.* 31(2): 415-421.

Sanchis-Gomar, F., Cortell-Ballester, J., Pareja-Galeano, H., Banfi, G, & Lippi, G. 2013. Hemoglobin point-of-care testing: the HemoCue system. *Journal of Lab Automation.* 18(3): 198-205.

Sandri, M. 2013. Protein breakdown in muscle wasting: Role of autophagy-lysosome and ubiquitin-proteasome. *The International Journal of Biochemistry and Cell Biology.* 45: 2121-2129.

Sayers, S.P., & Clarkson, P.M. 2001. Force recovery after eccentric exercise in males and females*. European Journal of Applied Physiology.* 84: 122–126.

Schaal, K., Meur, Y., Louis, J., Filliard, J. R., Hellard, P., Casazza, G., & Hausswirth, C. 2014. Whole-body cryostimulation limits overreaching in elite synchronized swimmers. *Medicine and Science in Sports and Exercise.* 47(7): 1416-1425.

Scherr, J., Wolfarth, B., Christle, J. W., Pressler, A., Wagenpfeil, S., & Halle, M. 2013. Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. *European Journal of Applied Physiology.* 113(1): 147-155.

Schoenfeld, B. J. 2010. The mechanisms of muscle hypertrophy and their application to resistance training. *Journal of Strength and Conditioning Research.* 24(10): 2857-2872.

Seitz, L. B., Reyes, A., Tran, T. T., Saez de Villarreal, E., & Haff, G. G. 2014. Increases in lower-body strength transfer positively to sprint performance: a systematic review with meta-analysis. *Sports Medicine.* 44(12): 1693-1702.

Selfe, J., Alexander, J., Costello, J. T., May, K., Garratt, N., Atkins, S., Dillon, S., Hurst, H., Davison, M., Przybyla, D., Coley, A., Bitcon, M., Littler, G. & Richards, J. 2014. The effect of three different (-135°C) whole body cryotherapy exposure durations on elite rugby league players. *Plos One.* 29;9(1): e86420.

Senne, J. A. 2016. Examination of gender equity and female participation in sport. *The Sport Journal.*

Sewright, K. A., Hubal, M. J., Kearns, A., Holbrook, M. T., Clarkson, P. M. 2008. Sex differences in response to maximal eccentric exercise*. Medicine and Science in Sports and Exercise*. 40(2): 242-251.

Skein, M., Wingfield, G., Gale, R., Washington, T. L., & Minett, G. M. 2018. Sleep quantity and quality during consecutive day heat training with the inclusion of cold-water immersion recovery. *Journal of Thermal Biology.* 74: 63-70.

Smith, L. L., McKune, A. J., Semple, S. J., Silbanda, E., Steel, H. & Anderson, R. 2007. Changes in serum cytokines after repeated bouts of downhill running. *Applied Physiology, Nutrition and Metabolism.* 32: 233-240.

Stanek, A., Sieroń, A., Cieślar, G., Matyszkiewicz, B., & Rozmus-Kuczia, I. 2005. The impact of whole-body cryotherapy on parameters of spinal mobility in patients with ankylosing spondylitis. *Ortopedia, Traumatologia, Rehabilitajca.* 7(5): 549-554.

Stephens, J. M., Halson, S. L., Miller, J., Slater, G. J., Chapman, D. W., & Askew, C. D. 2018. Effect of body composition on physiological responses to cold-water immersion and the recovery of exercise performance. *International Journal of Sports Physiology and Performance.* 13(3): 382-389.

Stocks, J. M., Taylor, N. A., Tipton, M.J., & Greenleaf, J. E. 2004. Human physiological responses to cold exposure. *Aviation, Space and Environmental Medicine.* 75(5): 444-457.

Stolen, T., Chamari, K., Castagna, C., & Wisløff, U. 2005. Physiology of soccer: an update*. Sports Medicine.* 35(6): 501-536.

Straburzyńska-Lupa, A, Kasprzak, M. P., Romanowski, M. W., Kwaśniewska A, Romanowski, W., Iskra, M, & Rutkowski R. 2018. The effect of whole-body cryotherapy at different temperatures on proinflammatory cytokines, oxidative stress parameters, and disease activity in patients with ankylosing spondylitis. *Oxidative Medicine and Cellular Longevity* 3;2018: 2157496.

Szczepańska-Gieracha, J., Borsuka, P., Pawika, M., & Rymaszewskaa, J. 2014. Mental state and quality of life after 10 session whole-body cryotherapy. *Psychology, Health & Medicine*. 19(1): 40–46.

Tabben, M., Ihsan, M., Ghoul, N., Coquart, J., Chaouachi, A., Chaabene, H., Tourny, C., & Chamari, K. 2018. Cold water immersion enhanced athletes' wellness and 10m short sprint performance 24h after a simulated mixed martial arts combat. *Frontiers in Physiology*. 9: 1542.

Tanda, G. 2015. The use of infrared thermography to detect the skin temperature response to physical activity*. Journal of Physics Conference Series. 655, 33rd UIT.*

Tanimoto, M., Kawano, H., Gando, Y., Sanada, K., Yamamoto, K., Ishii, N., Tabata, I., & Miyachi M. 2009. Low-intensity resistance training with slow movement and tonic force generation increases basal limb blood flow. *Clinical Physiology and Functional Imaging.* 29(2): 128-135.

Tee, J.C; Bosch, A. N., & Lambert, M. I. 2007. Metabolic consequences of exercise-induced muscle damage. *Sports Medicine*. 37: 827–836,

Thomas, K., French, D., & Hayes, P. R. 2009. The effect of two plyometric training techniques on muscular power and agility in youth soccer players. *Journal of Strength and Conditioning Research.* 23(1): 332-335.

Tighe, P., Negm, O., Todd, I., & Fairclough, L. 2013. Utility, reliability and reproducibility of immunoassay multiplex kits. *Methods.* 61(1): 23-29.

Turner, A. 2011. The science and practice of periodization: A brief review. *Strength and Conditioning Journal*. 33(1): 34-46.

Twist, C. & Highton, J. 2013. Monitoring fatigue and recovery in rugby league players*. International Journal of Sports Physiology and Performance*. 8: 467-474.

Urhausen, A., Gabriel, H., & Kindermann, W. 1995. Blood hormones as markers of training stress and overtraining. *Sports Medicine*. 20(4): 251-276.

Van Hooren, B. & Zolotarjova, J. 2017. The difference between countermovement and squat jump performances: A review of underlying mechanisms with practical applications. *Strength and Conditioning Research.* 31(7): 2011-2020

Vecchio, L., D., Daewoud, H., & Green, S. 2018. The health and performance benefits of the squat, deadlift and bench press. *MOJ Yoga and Physical Therapy*. 3(2): 40–47.

Versey, N. G., Halson, S. L., & Dawson, B. T., 2013. Water immersion recovery for athletes: effect on exercise performance and practical recommendations. *Sports Medicine*. 43(11): 1101-1130.

Vieira, A., Bottaro, M., Ferreira-Junior, J. B., Vieira, C., Cleto, V. A., Cadore, E. L., Simões, H. G., Carmo, J. D., & Brown, L. E. 2015. Does whole-body cryotherapy improve vertical jump recovery following a high-intensity exercise bout? *Open Access Journal of Sports Medicine.* 6: 49–54.

Vieira, A., Siqueira, A. F., Ferreira-Junior, J. B., do Carmo, J., Durigan, J. L., Blazevich, A., & Bottaro, M. 2016. The effect of water temperature during cold-water immersion on recovery from exercise-induced muscle damage*. International Journal of Sports Medicine*. 37(12): 937-943.

Vieira Ramos, G., Pinheiro, C. M., Messa, S. P., Delfino, G. B., Marqueti, R., Salvini, T., & Durigan, J. L. 2016. Cryotherapy reduces inflammatory response without altering muscle regeneration process and extracellular matrix remodeling of rat muscle. *Scientific Reports*, 6, 18525.

Walsh, N. P., Halson, S. L., Sargent, C., Roach, G. D., Nédélec, M., Gupta, L., Leeder, J., Fullagar, H. H., Coutts, A. J., Edwards, B. J., Pullinger, S. A., Robertson, C. M., Burniston, J. G., Lastella, M., Le Meur, Y., Hausswirth, C., Bender, A. M., Grandner, M. A., & Samuels, C. H. 2020. Sleep and the athlete: narrative review and 2021 expert consensus recommendations. *British Journal of Sports Medicine.* 55: 356-368.

Warren, G. L., Lowe, D. A., & Armstrong, R. B. 1999. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Medicine.* 27(1): 43-59.

Weltman, A., Snead, D., Stein, P., Seip, R., Schurrer, R., Rutt, R., & Weltman, J. 1990. Reliability and validity of a continuous incremental treadmill protocol for the determination of lactate threshold, fixed blood lactate concentrations, and VO_2 max. *International Journal of Sports Medicine*. 11(1): 26-32.

Westerlund, T., Oksa, J., Smolander, J., & Mikkelsson, M. 2009. Neuromuscular adaptation after repeated exposure to whole-body cryotherapy (-110°C). *Journal of Thermal Biology.* 34: 226–231.

White, G. E., & Wells, G. D. 2013. Cold-water immersion and other forms of cryotherapy: physiological changes potentially affecting recovery from high-intensity exercise. *Extreme Physiology & Medicine.* 2:26.

Whyte, G. P., George, K., Shave, R., Middleton, N., & Nevill, A. M. 2008. Training induced changes in maximum heart rate. *International Journal of Sports Medicine.* 29(2): 129-133.

Wiewelhove, T., Fernandez-Fernandez, J., Raeder, C, Kappenstein J, Meyer, T., Kellmann M, Pfeiffer, M., & Ferrauti, A. 2016. Acute responses and muscle damage in different high-intensity interval running protocols. *Journal of Sports Medicine and Physical Fitness.* 56(6): 606-615.

Wilson, L. J., & Cockburn, E., Paice, K., Sinclair, S., Faki, T., Hills, F. A., Gondek, M. B., Wood, A., & Dimitriou, L. 2018. Recovery following a marathon: a comparison of cold water immersion, whole body cryotherapy and a placebo control. *European Journal of Applied Physiology.* 118(1): 153-163.

Wilson, L. J., Dimitriou, L., Hills, F. A., Gondek, M. B, Cockburn, E. 2019. Whole body cryotherapy, cold water immersion, or a placebo following resistance exercise: a case of mind over matter? *European Journal of Applied Physiology*. 119(1): 135-147.

Wong, P. L., Chaouachi, A., Chamari, K., Dellal, A., & Wisloff, U. 2010. Effect of pre-season concurrent muscular strength and high-intensity interval training in professional soccer players. *Journal of Strength and Conditioning Research.* 24(3): 653-660.

Wozniak, A., Wozniak, B., & Drewa, G. 2007. The effect of whole body cryostimulation on lysosomal enzyme activity in kayakers during training. *European Journal of Applied Physiology.* 100: 137-142.

Wozniak, A., Wozniak, B., Drewa, G., & Mila-Kierzenkowska, C. 2007. The effect of whole-body cryostimulation on the prooxidant-antioxidant balance in blood of elite kayakers after training. *European Journal of Applied Physiology.* 101(5): 533-537.

Yamane, M., Teruya, H., Nakano, M., Ogai, R., Ohnishi, N., & Kosaka, M. 2006. Post-exercise leg and forearm flexor muscle cooling in humans attenuates endurance and resistance training effects on muscle performance and on circulatory adaptation. *European Journal of Applied Physiology.* 96: 572–580.

Yin Lau, W., Muthalib, M., & Nosaka, K. 2013. Visual analog scale and pressure pain threshold for delayed onset muscle soreness assessment. *Journal of Musculoskeletal Pain.* 21(4): 320-326.

Yoon, E. J., & Kim, J. 2020. Effect of body fat percentage on muscle damage induced by high-intensity eccentric exercise. *International Journal of Environmental Research and Public Health*. 17(10): 3476.

Young, W. B., Pryor, J. F., & Wilson, Greg. 1995. Effect of instructions on characteristics of countermovement and drop jump performance. *Journal of Strength and Conditioning Research*: 9(4): 232-236.

Young, A. J., Sawka, M. N., & Pandolf, K. B. 1996. Physiology of cold exposure. *Nutritional Needs in Cold and High-Altitude Environments: Applications for Military Personnel in Field Operations. National Academies Press.* 127-148.

Young, W. B. 2006. Transfer of strength and power training to sports performance. *International Journal of Sports Physiology and Performance.* 1(2): 74-83.

Zembron-Lacny, A., Morawin, B., Wawrzyniak-Gramacka, E., Gramacki, J., Jarmuzek, P., Kotlega D., & Ziemann E. 2020. Multiple cryotherapy attenuates oxi-inflammatory response following skeletal muscle injury. *International Journal of Environmental Research and Public Health.* 17(21): 7855.

Ziemann, E., Olek, R. A., Kujach, S., Grzywacz, T., Antosiewicz, J., Garsztka, T., & Laskowski, R. 2012. Five-day whole-body cryostimulation, blood inflammatory markers, and performance in high-ranking professional tennis players. *Journal of Athletic Training.* 47(6): 664-672.

Ziemann, E., Olek, R. A., Grzywacz, T., Kaczor, J. J., Antosiewicz, J., Skrobot, W., Kujach, S., & Laskowski, R. 2013. Whole-body cryostimulation as an effective way of reducing exercise-induced inflammation and blood cholesterol in young men. *European Cytokine Network*. 25: 14-23.

Appendices

THE UNIVERSITY OF NORTHAMPTON		*Risk Assessment*	
Location:	Chris Moody Sports Injury and Rehabilitation Centre, Moulton Everdon Building, Park Campus, University of Northampton, Northampton (until summer 2018) Sports Science laboratories, Waterside Campus, University of Northampton (from August 2018)	**Date:**	08/04/2016

Hazard	Who might be harmed and how	Existing Control Measures	Additional Control Measures	Risk Level
Frostbite / cold-related injuries	Participants, due to cold exposure in cryogenic chamber.	Duration of entire treatment is strictly limited to 3 minutes (alarm will sound and machine will stop if duration exceeds this). Participants required to wear specified clothing prior to chamber treatment – e.g. headband, gloves and socks - to cover extremities. Participants required to remove jewellery and towel dry to remove sweat. Participants asked to mobilize joints whilst inside chamber and to avoid physical contact with the chamber walls for long periods.	Participants will be closely supervised and monitored. Lining of chamber walls to be maintained on regular basis to minimize harmful effects of low temperatures on skin. The chamber will be maintained frequently and checked for potential leakages of cryogenic gas.	3
Feeling faint, light headed, dizzy or nauseous. Probable discomfort.	Participants, due to exercise exertion and possible overtraining. Strenuous exercise bouts include maximal	Medical and physical activity readiness questionnaires will ensure participants' suitability to take part, along with measures such as resting blood pressure. Participants with existing heart conditions will be contra-indicated from taking part.	Fluids will be provided to maintain hydration levels. Downhill running intensity will be set relatively low and pre-determined following initial screening to ensure suitability to take part.	4

Hazard	Who might be harmed and how	Existing Control Measures	Additional Control Measures	Risk Level
	aerobic capacity testing and downhill running. Inclusion of relatively unfit and older participants will be noted.	Participants will provide written consent form prior to taking part. Speed and intensity will be progressed gradually for maximal aerobic capacity test, ensuring participants are thoroughly warmed up prior to high exertion. Participants will be closely monitored throughout exercise bouts and frequently provide feedback on how they feel. Participants informed they can stop exercise at any time	Downhill running duration may be set considerably lower for older/less fit subjects, pending findings. Participants will be reminded to avoid any physical exertion or alcohol intake for 24 hours prior to exercise sessions or blood collections.	
Feeling faint / light headed, dizzy or nauseous. Possible discomfort.	Participants, due to blood collections by venepuncture	Pre-existing cardiovascular conditions are a contra-indication for participating and undergoing blood collections. Participants to be as relaxed and comfortable as possible. Instructions provided to participants to avoid strenuous activity and alcohol prior to testing trials. Venepuncture will be performed by a qualified phlebotomist.	Maintain hydration status of participants – provide fluids before and after exercise. Consider nutritional status. Phlebotomy training to occur before commencing data collection. Consider positioning of participant during blood collections – e.g. lay supine on bed. Consider ambient temperature – try to ensure it is as close to 20C as possible.	4
Possible contamination	Participants and researcher, from use of facemask and components for expired gas analysis	Facemask and gas analysis components cleaned and treated thoroughly with disinfectant (Milton tablets dissolved in solution at recommended dosage) prior to and after participant use for sufficient duration.		2

Hazard	Who might be harmed and how	Existing Control Measures	Additional Control Measures	Risk Level
Possible contamination	Researcher and participant, from blood collection by venepuncture and handling procedures. Potential spilling of blood and spread of blood-borne diseases.	Gloves will be worn for all blood sampling procedures. Basic personal hygiene adhered to. Alcohol wipes to sterilise sites of blood collections. Venepuncture will be performed by a qualified phlebotomist. Blood vacutainer tubes will be appropriately labelled. Blood centrifugation will occur within 2 hours after sample collection. Blood spills wiped up immediately. Waste to be cleaned and disposed of in clinical waste. Participants screened for diseases/conditions (e.g. blood-borne) via questionnaire prior to trials.	Phlebotomy training to occur before commencing data collection. Researcher to be vaccinated against hepatitis B.	2
Muscle injury – e.g. strain	Participants, due to exercise exertion (running, resistance exercises – e.g. weights, muscle torque assessment)	Medical and physical activity readiness questionnaires will ensure participants' suitability to take part.	Participants will be reminded to avoid any physical exertion or alcohol intake for 24 hours prior to exercise sessions. Most exercise protocols will be at relatively low intensities. High intensity exercises will be preceded by warm ups / intensity progressions.	4

Hazard	Who might be harmed and how	Existing Control Measures	Additional Control Measures	Risk Level
Injuries – e.g. wounds, cuts, bruises	Participants, due to falling, for instance when exiting cryogenic chamber. Participants and researcher due to sharp needles for blood collections by venepuncture	Participants will be closely monitored and reminded to watch their step when they exit the chamber at the end of their treatment. Blood needles to be disposed of in sharps waste bin positioned next to researcher immediately after blood sample withdrawals. All trip hazards to be accounted for (e.g. extensive cables) and lab areas to be cleared.	Participants will also be familiarized to the cryogenic chamber and explained all safety precautions prior to treatment.	3
Electric shock	Participants and researcher, due to use of electrical appliances – e.g. cryogenic chamber, online gas analysis	Lab manuals available for procedures and electrical equipment (e.g. Douglas Bag analysis) Electrical equipment tested by Moulton College and University of Northampton staff. Large quantities of water will not be handled close to appliances.	Researcher will undergo necessary training and familiarity with electrical equipment. Electrical equipment will be visually checked before each use and switched off after use Cryogenic chamber will be maintained and checked for potential electrical faults on regular basis.	3
Gas leaking (oxygen, carbon dioxide from canisters; nitrogen from cryotherapy chamber)	Participants and researcher. Possible irritation of eyes and throat if excess gas leakage occurs.	Low risk due to small canister size and small gas volume (very unlikely to exceed WEL) All gas canisters thoroughly checked, ensuring expiry dates are not exceeded. Standard operating procedures in place for cryotherapy chamber at Moulton College Injury Rehabilitation Centre.	All canisters will be closed securely after use.	2

Hazard	Who might be harmed and how	Existing Control Measures	Additional Control Measures	Risk Level
Discomfort associated with confined spaces	Participants, due to confined space in cryogenic chamber, possible close proximity with other participants due to chamber sharing.	All participants will be screened via medical health questionnaire prior to taking part. Claustrophobia is a contra-indication for participation. Participants will be familiarised to the cryogenic chamber before commencing experimental trials.	Simultaneous testing of participants is more economical and might be an occasional occurrence. Participants will be introduced to each other and encouraged to communicate with each other where relevant. They will be explained that other participants will be undergoing same procedure.	2

RISK MATRIX

RISK				
Severity	3	3	6	9
	2	2	4	6
	1	1	2	3
		1	2	3
		Likelihood		

SEVERITY	
3	Major injury
2	Minor injury
1	Negligible impact

LIKELIHOOD	
3	Highly Likely
2	Possible
1	Improbable

University of Northampton	***Risk Assessment for COVID-19*** *(following guidelines of government and Physiological Society)* Date: 01/08/2020

Activity:	Data collection for PhD project by Adnan Haq.

Hazard	**Who might be harmed**	**Existing Control Measures** (What are you already doing already to manage the risks?)	**Risk Level**
Lab work - Possible direct transmission of Covid-19 via airborne particles or indirectly by physical contact of affected lab surfaces Possible transmission of Covid-19 via means outside of lab.	Participants and researcher	All participants and researchers are to be treated as if they are infected. All participants and researcher to have temperature measured upon lab entry (Braun Thermoscan thermometers). **Regular handwashing** by both researcher and participant, from arrival in laboratory through to exit following session finish. Hands to be washed thoroughly by researcher after each contact with participant. **Social distancing** (2 metres+) in the laboratory between participant and researcher and from other possible researchers. A limit on the number of people permitted in the lab at a given time may be necessary. Where this is not possible, social distancing must be maintained. All PhD study sessions will involve just one participant at a time, with no groups involved. Precautions to be taken (detailed below) when social distancing not possible during close contact with participants – e.g.	4

Hazard	Who might be harmed	Existing Control Measures (What are you already doing already to manage the risks?)	Risk Level
Same as above		blood samples, applying face mask for maximal exercise testing and assessing leg strength on the Biodex dynamometer. Necessary **protective equipment** to be required and worn: - Includes researcher wearing **personalised lab coat** - **Face mask x 60 (30 x surgical mask FFP2)** during laboratory trials to cover researcher and participants for remaining lab trials. All facemasks are disposable. - **Face shield x 2** – re-usable, may be more practical and comfortable than masks and provides added protection. Any EN166 certified shield will suffice. https://www.arco.co.uk/products/340700?s=1&BV_SessionID=@@@@0609037579.1594048837@@@@&BV_EngineID=ccccadhmgkdmkhfcflgcefkdfggdgjf.0 - Goggles are necessary if face shields are not used, particularly during close contact. - **Nitrile gloves x 200** are necessary during blood samples or when handling body fluids e.g. removal of facemask after VO2 max test. Gloves must be discarded following single use. Regular handwashing/alcohol gel application to be adhered to otherwise. Proper preparation and setting up of laboratory prior to each session, ensuring all surfaces are clean and clear from excess clutter. Lab spaces must be kept as clear as possible. All surfaces and equipment thoroughly cleaned and disinfected before and after use (e.g. biodex with straps and attachments, cortex, treadmill etc). Where possible, laboratory equipment used by researcher shall not be shared with other researchers during a given trial/session.	4

Hazard	Who might be harmed	Existing Control Measures (What are you already doing already to manage the risks?)	Risk Level
		Participants to apply measuring equipment without researcher intervention where possible (e.g. heart rate monitors). Participants and researcher are required to enter laboratory with fresh clothes (participants need to change clothes if they are coming straight from work). Clothes to be changed between each lab session. Participants are required to bring their own water and towels. Ample supply for alcohol gels, disinfectant sprays and paper towels to be ensured in the laboratory at all times. Regular ventilation of labs using fresh air to dilute respiratory particles. Use of plastic screens may be necessary on occasions to separate researchers and participants, particularly where multiple studies are being performed simultaneously. All adverse events specific to COVID-19 - including symptoms, positive tests – to be recorded. Participants to monitor and report symptoms for 14 days prior to and throughout study (weekly monitoring for training study). Identification of symptoms to report based on government guidelines and to be declared before/during study. Close contact with others to also be monitored and minimised where possible. Paperwork to be minimised as far as possible. All preliminary forms to be sent and filled electronically (e.g. consent form). Participants to fill in visual analogue scales (e.g. muscle soreness) on personal devices/smartphones and send separately. Government guidelines to be followed and checked on a regular basis.	

Appendix 2 - Contra-indications for Whole Body Cryotherapy treatment

Hypertension

Cardiovascular disease, including coronary artery disease, arrhythmia, arterial valve stenosis and mitral valve stenosis

Open wounds and ulcers

Central Nervous System diseases, including neuropathy

Claustrophobia

Cancers

Hypothermia/Cold intolerance

Mental disorders

University of Northampton

Moulton COLLEGE

Whole Body Cryotherapy for Sports Recovery

Medical and Physical Activity Readiness Questionnaire - CONFIDENTIAL

Name: _____ Date:...../...../.....

Date of birth:...../...../.....

PAST HISTORY (i.e. Have you ever had?) Please tick appropriate box. If yes, please give details

	No	Yes
Rheumatic fever	[ ]	[ ]
High blood pressure	[ ]	[ ]
Any heart trouble	[ ]	[ ]
Any blood vessel disorders (e.g. Varicose veins, Raynauds)	[ ]	[ ]
Anaemia	[ ]	[ ]
Lung disease	[ ]	[ ]
Asthma	[ ]	[ ]
Kidney or liver disease	[ ]	[ ]
Diabetes	[ ]	[ ]
Epilepsy	[ ]	[ ]
Peptic ulcer	[ ]	[ ]
Any blood clotting disorders	[ ]	[ ]
Any neural disorders	[ ]	[ ]
Any form of cancer	[ ]	[ ]

FAMILY HISTORY (i.e. Have any of your immediate family had?) If yes, please give details

	No	Yes
Heart disease	[ ]	[ ]
High blood pressure	[ ]	[ ]
High cholesterol	[ ]	[ ]
Stroke	[ ]	[ ]
Diabetes	[ ]	[ ]
Heart operations	[ ]	[ ]
Any blood clotting disorders	[ ]	[ ]

PRESENT SYMPTOMS (i.e. Have you recently had?) If yes, please give details

	No	Yes
Chest pain or discomfort	[ ]	[ ]
Shortness of breath	[ ]	[ ]
Heart palpitations or skipped heart beats	[ ]	[ ]
Coughing of blood	[ ]	[ ]
Dizzy spells	[ ]	[ ]

Fever	[ ]	[ ]
Back pain	[ ]	[ ]
Recurrent injury	[ ]	[ ]
Are you presently taking any medication?	[ ]	[ ]
Do you have any allergies?	[ ]	[ ]
Have you had any surgery in the last 12 months?	[ ]	[ ]
Any other medical problems not already indicated?	[ ]	[ ]

Please list any current prescription and non-prescription medications:

_____

_____

Could you please specify if you have recently been diagnosed with any blood borne diseases (e.g. malaria, HIV)

Do you currently smoke?: Yes No

If so what and how many?.....

Have you ever quit smoking? Yes No

If yes, for how many years did you smoke?.....

How much alcoholic beverage do you roughly consume each week? (guide: pint of beer/cider – 3 units, standard glass of wine – 2 units, single shot of spirit – 1 unit)

25+ units [ ] 20-25 units [ ] 15-19 units [ ]
10-14 units [ ] 5-9 units [ ] 1-4 units [ ]
I do not consume alcohol [ ]

How much and what type of caffeinated beverage do you consume per day?

Coffee..... Tea..... Soft Drinks.....

ACTIVITY LEVEL EVALUATION

Do you engage in physical activity? Yes No

If so what type/s? .....
How many days per week? .....
How much time per day?

Less than 15 minutes [ ] 15 to 30 minutes [ ] 30 to 60 minutes [ ] More than 60 minutes [ ]

Please answer the following:	No	Yes
If yes, please give details		
Do you ever experience shortness of breath during exercise?	[ ]	[ ]
Do you ever experience chest discomfort during exercise?	[ ]	[ ]
If so, does it go away with rest?	[ ]	[ ]
Do you lose your balance because of dizziness or do you ever lose consciousness?	[ ]	[ ]
Do you know of any other reason why you should not do exercise?	[ ]	[ ]

If you answered yes, to any of the above, please give details:

How would you describe your state of well-being at this time?

Very good	[ ]
Good	[ ]
Neither good nor bad	[ ]
Poor	[ ]
Very poor	[ ]

Having understood the procedures and exercise demands required by the study, is there anything which may stop you from completing the assessments?

Signed …… Date……

STATEMENT OF INFORMED CONSENT

Study title: Evaluating the effects of whole body cryotherapy treatment for sports recovery/training.

Name of assessor: ADNAN HAQ

Name of participant: .....

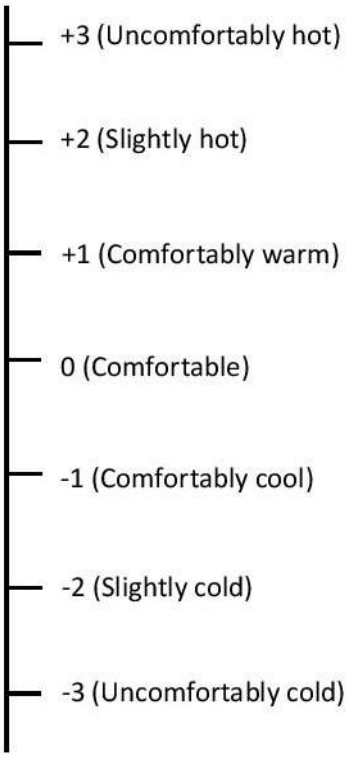
Date of assessment: ...../...../.....

The nature and content of the study have been explained to me and I am willing to participate fully. I confirm that I have read the associated participant information sheets and am fully aware of what the research involves, including the possible risks. I reserve, however, the right to withdraw from the assessment at any stage or to omit any element of the assessment.	Yes / No
I confirm that I have been given the opportunity to ask questions.	Yes / No
I am aware that I am in good health and any medication that I am taking has been recorded on the relevant questionnaire. To the best of my knowledge, there is nothing that should prevent me from successfully completing the study protocol.	Yes / No
I am aware that the information obtained during the study will be treated as confidential and will not be released to any unauthorised personnel without my expressed written consent. The information, however, may be used for statistical or scientific purposes with my right of privacy maintained. I am also aware that I have the right to choose to receive a summary of the research findings.	Yes / No
I agree to participate in the study.	Yes / No

Surname:..... First name(s): .....

Signature: .....

Appendices 5-7 – Visual Analogue Scales for muscle soreness, thermal comfort and wellbeing



Appendix 8 – Rating of Perceived Exertion Scale

Borg Rating of Perceived Exertion

6 No exertion at all
7
Extremely light
8
9 Very light
10
11 Light
12
13 Somewhat hard
14
15 Hard (heavy)
16
17 Very hard
18
19 Extremely hard
20 Maximal exertion

Whole Body Cryotherapy for Sports Recovery

Sleep Questionnaire

Participant name:

Using the descriptive scale, how would you rate your overall sleep quality for each night?

5–Very good 4-Good 3- Average

2-Poor 1-Very poor

Three nights before main downhill run trial:

5 4 3 2 1

Two nights before main trial:

5 4 3 2 1

Night before main trial:

5 4 3 2 1

Night following main trial:

5 4 3 2 1

Pilot Study 1 – Muscle Torque Variability and Downhill Running

Participant Information Sheet

Thank you for choosing to take part in one of my pilot studies. You will be asked to report to the laboratory 6 times. There are two trials. The first trial involves 4 sessions on consecutive days. The second trial involves 2 sessions on consecutive days. There will be at least a 7 day period between the two trials. Testing will take place at the Everdon sports building at the University of Northampton Park Campus (postcode NN2 7AL) *unless specified otherwise*. The procedures and durations for each trial and session are outlined as follows:

Trial 1

Day 1 session (total duration – 40 mins approx):

1. Measures of blood pressure, body mass and height

2. Assessment of maximal leg muscle strength using torque dynamometer (see diagram) following familiarisation and muscle soreness measure.

Day 2 session (total duration – 15 mins approx)

Leg muscle strength assessment, muscle soreness

Day 3 session (total duration – 60 mins approx):

1. Maximal leg muscle strength assessment, muscle soreness

2. Downhill running on treadmill for 20 minutes with heart rate monitor attached.

**NB – Downhill run for trial 1 will occur on treadmill in Everdon Sports Building or Chris Moody Centre at Pitsford Site of Moulton College (postcode NN3 7QL, <10 min drive from University Park Campus).*

Day 4 session (total duration – 15 mins approx)

Maximal leg muscle strength assessment, muscle soreness

Trial 2

Day 1 session (total duration – 60 mins approx)

1. Maximal leg muscle strength assessment, muscle soreness

2. Downhill running on treadmill for 20 minutes with heart rate monitor attached. *

**NB – Downhill run for trial 1 will occur on treadmill in Everdon Sports Building or Chris Moody Centre at Pitsford Site of Moulton College (postcode NN3 7QL, <10 min drive from University Park Campus).*

Day 2 session (total duration – 15 mins approx)

Maximal leg muscle strength assessment, muscle soreness

Please refrain from alcohol and strenuous physical activity for the duration of testing.

What are the possible disadvantages and risks of taking part?

The risks involved mainly relate to your exercise tolerance and discomfort. Whilst you are expected to be reasonably fit and healthy, it is likely that you will feel sore for 2-3 days following the downhill running procedure. You will be closely supervised throughout all testing. You have the right to withdraw from the study at any point.

Further information and contact details:

If you have any concerns or queries, you may contact the following:

Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

Bill Ribbans, supervisory team, billribbs@uk-doctors.co.uk;

Tony Baross, supervisory team, Anthony.Baross@northampton.ac.uk

Pilot Study 2 – Whole Body Cryotherapy and Blood Sampling

Participant Information Sheet

Thank you for choosing to take part in one of my pilot studies. You will be asked to report to the laboratory 3 times on consecutive days. Testing will take place at the Chris Moody Centre at Pitsford Site of Moulton College (postcode NN3 7QL)

The procedures and durations for each session are outlined as follows:

Day 1 session (total duration – 30 mins approx):

1. Instructions and explanation of procedures. Questionnaire, consent form.

2. Blood sample (via venepuncture) followed by familiarisation of cryogenic chamber.

Day 2 session (total duration – 10 mins approx)

Blood sample

Day 3 session (total duration – 40 mins approx):

1. Measures of blood pressure, height and body mass

2. Whole body cryotherapy treatment – 3 mins duration (see diagram below)

3. Blood sample

Please refrain from alcohol and strenuous physical activity for the duration of testing. You are also encouraged to arrive to each session in a well hydrated and well fed state.

What are the possible disadvantages and risks of taking part?

The risks involved with exposure to cold air are mainly related to cold intolerance and discomfort. Steps will be taken to ensure your safety throughout, such as wearing specialised clothing to cover your extremities and you will be supervised the entire time. Other minor risks include small changes in blood pressure, cold allergic reactions and claustrophobia. Blood collection procedures may be associated with feeling faint or light-headed. It is important that you inform the researchers of any discomfort.

You have the right to withdraw from the study at any point.

Further information and contact details:

If you have any concerns or queries, you may contact the following:

Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

Moulton College, Moulton, Northamptonshire, NN3 7RR

Bill Ribbans, supervisory team, billribbs@uk-doctors.co.uk

Tony Baross, supervisory team, Anthony.Baross@northampton.ac.uk

Pilot Study 3

Participant Information Sheet

Thank you for choosing to take part in one of my pilot studies. The main purpose of this study is to compare the impact of two possible muscle damaging exercise protocols to be used later on in my research. The two exercise protocols are downhill running for 30 minutes and strength training exercises targeting the lower body. You will be asked to report to the laboratory 5 times in total. The first session involves familiarisation and tests for strength training exercises. The second session will occur at least 5 days later. The third session will occur 24 hours after the second. The final two sessions will occur at least 5 days later and be a repeat of sessions 2 and 3, with the alternative exercise protocol being performed. Testing will take place at the Everdon sports building at the University of Northampton Park Campus (postcode NN2 7AL).

The procedures and durations for each session are outlined as follows:

Session 1 (Familiarisation and One rep max tests, total duration – 45 mins approx):

1. Measures of blood pressure, body mass and height

2. Familiarisation to muscle leg strength assessment using torque dynamometer (see diagram below)

3. Testing one repetition maximum for weight training exercises:

- Weighted lunge

- Barbell squat (see diagram below)

- Leg extension

Session 2 (total duration – 60 mins approx)

1. Maximal leg muscle strength assessment, muscle soreness

2. Exercise protocol – either strength training routine (13 sets, 5 different exercises) OR downhill running on treadmill for 30 minutes with heart rate monitor attached.

Session 3 (total duration – 15 mins approx):

Maximal leg muscle strength assessment, muscle soreness

Session 4 (total duration – 60 mins approx)

1. Maximal leg muscle strength assessment, muscle soreness

2. Exercise protocol – either strength training routine (13 sets, 5 different exercises) OR downhill running on treadmill for 30 minutes with heart rate monitor attached.

Session 5 (total duration – 15 mins approx):

Maximal leg muscle strength assessment, muscle soreness

Please refrain from alcohol and strenuous physical activity for the duration of testing.

What are the possible disadvantages and risks of taking part?

The risks involved mainly relate to your exercise tolerance and discomfort. Whilst you are expected to be reasonably fit and healthy, it is likely that you will feel sore for 1-2 days following the strength training and running exercises. In addition, there is a possibility of sustaining injury when performing exercises (e.g. muscle strain). You will be supervised throughout all testing. You have the right to withdraw from the study at any point.

Further information and contact details:

If you have any concerns or queries, you may contact the following:

Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

Moulton College, Moulton, Northamptonshire, NN3 7RR

Bill Ribbans, supervisory team, billribbs@uk-doctors.co.uk

Tony Baross, supervisory team, Anthony.Baross@northampton.ac.uk

Pilot Study 3 (re-trial)

Participant Information Sheet

Thank you for choosing to take part in one of my pilot studies. The main purpose of this study is to examine the impact of a possible muscle damaging exercise protocol to be used later on in my research. This exercise protocol involves downhill running for 30 minutes. You will be asked to report to the laboratory twice with the two sessions occurring 24 hours apart. Testing will take place at the Everdon sports building at the University of Northampton Park Campus (postcode NN2 7AL).

The procedures and durations for each session are outlined as follows:

Session 1 (total duration – 60 mins approx)

1. Muscle soreness, maximal leg muscle strength assessment.

2. Exercise protocol – Downhill running on treadmill for 30 minutes with heart rate monitor attached.

Session 2 (total duration – 15 mins approx):

Muscle soreness, maximal leg muscle strength assessment,

Please refrain from alcohol and strenuous physical activity for the duration of testing.

What are the possible disadvantages and risks of taking part?

The risks involved mainly relate to your exercise tolerance and discomfort. Whilst you are expected to be reasonably fit and healthy, it is likely that you will feel sore for 1-2 days following the strength training and running exercises. In addition, there is a possibility of sustaining injury when performing exercises (e.g. muscle strain). You will be supervised throughout all testing. You have the right to withdraw from the study at any point.

Further information and contact details:

If you have any concerns or queries, you may contact the following:

Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

Moulton College, Moulton, Northamptonshire, NN3 7RR

Bill Ribbans, supervisory team, billribbs@uk-doctors.co.uk

Tony Baross, supervisory team, Anthony.Baross@northampton.ac.uk

Title of Study

The influence of age and body fat content on the effectiveness of Whole Body Cryotherapy (WBC) treatment on recovery from exercise.

Invitation and Purpose

You are being invited to participate in a research study examining the effects of age and body fat content on WBC treatment. Taking part is voluntary and you have the right to physically withdraw at any point if you wish. Should you wish to take part, you will be asked to sign a consent form. WBC involves cold exposure to treat a variety of conditions. WBC is suggested to improve your recovery from exercise. WBC has also been shown to promote wellbeing, as well as having beneficial effects on quality of sleep.

Whole body cryogenic chamber at the Chris Moody Centre, which contains cold dry gas. This has previously been used by sports clubs such as Leicester Tigers and Northampton Saints.

What will happen to me if I take part and how long with my participation take?

Your participation will consist of 3 trials on separate days. Trial 1 is likely to take approximately 90 minutes. Trial 2 is likely to take approximately 2 hours. Trial 3 is likely to take approximately 20 minutes. Trials 2 and 3 occur on consecutive days. You may be asked to perform these trials as a non-cryotherapy participant (this will be explained in person if this applies to you). You will be asked to arrive at the University Waterside Campus and to refrain from alcohol intake and strenuous activity for 24 hours and 48 hours respectively before each session.

You will be asked to complete a medical questionnaire. For your first trial, you will have several measures taken (see appendix for complete list of measurements). It is important that you meet the inclusion criteria and are free from conditions that could make the cryotherapy treatment unsafe. You will be explained all testing procedures to follow.

You will perform two exercise tests. The first involves treadmill running where you will have a face mask on and exercise at progressive intensities until exhaustion. The second will be an assessment of your leg muscle strength.

A few days after the initial testing, you will be asked to report to the University Waterside Campus for trial 2. Several measurements (see appendix) will be taken before and after exercise. You will be

asked to perform a treadmill downhill running session. Following the run, further measurements will be taken and you will transport to the Chris Moody Centre for the cryotherapy treatment. This treatment will occur 60 minutes following the treadmill run. The treatment involves exposure to cold dry gas for a duration of 3 minutes. You will be asked to move around inside the chamber and will be supervised throughout. If you are assigned as a control participant you will not undergo this cryotherapy treatment and will instead remain seated in the University Campus. The pre-treatment measures will be assessed on completion of the treatment (see trial 2 flow chart appendix). The next day (trial 3), you will be asked to perform the same leg muscle strength test outlined above.

What are the possible disadvantages and risks of taking part?

The risks involved with exposure to cold air are mainly related to cold intolerance and discomfort. Steps will be taken to ensure your safety throughout, such as wearing specialised clothing to cover your extremities and you will be supervised the entire time. Other minor risks include small changes in blood pressure, cold allergic reactions and claustrophobia. You will be asked to assess your own thermal comfort and wellbeing levels regularly and it is important that you inform the researcher of any discomfort. As with any participation in strenuous exercise, it is usual to feel tired and sore afterwards. It is also important to reduce risk of injury by ensuring you are properly rested before exercise sessions.

What will happen to the data collected?

All data collected will be kept strictly confidential and stored safely on a password protected personal file in accordance with the Data Protection Act 1998. Your research data will be kept anonymous and identified by initials. Once your data has been collected and analysed, they will be available for you to access at your request. The results will be shared among the project stakeholders and might be published in a journal. The individual findings will only be identified in reports or publications if you have provided your consent. Should you wish to withdraw, your data will not be used after 1 month following completion of your participation.

Further information and contact details:

If you have any concerns, queries or would like to make a complaint, you may contact the following:

Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

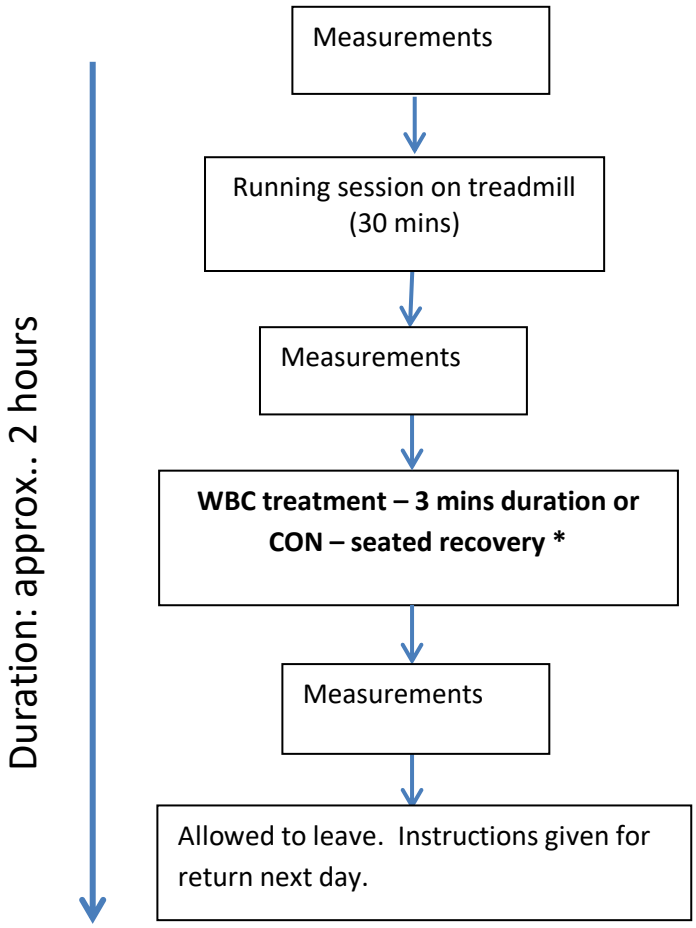
Moulton College, Moulton, Northamptonshire, NN3 7RR

Bill Ribbans, supervisory team, billribbs@uk-doctors.co.uk;

Chris Moody Sports Therapy and Injury Rehabiliation Centre - 01604 492 222, http://sportstherapy.moulton.ac.uk/Services/Cryotherapy

Appendix

Flow chart of procedures for trial 2 - Main experimental trial (a few days after trial 1):



WBC – Whole Body Cryotherapy

CON – Control / non-cryotherapy

*NB – Cryotherapy treatment to occur at Chris Moody Centre. All other procedures (e.g. running) occur at University Waterside Campus.

The following measures will be taken:

Trial 1: Blood pressure, body fat (skinfold), mass and height, VO2 max, muscle torque,

Trial 2: Tympanic temperatures, skin temperatures, muscle soreness, thermal comfort, wellbeing. Blood samples for creatine kinase and haemoglobin.

Trial 3: Muscle torque, muscle soreness, wellbeing blood creatine kinase, haemoglobin.

The nature and timings of these measures will be explained in more detail in person.

Title of Study

The effect of timing of Whole Body Cryotherapy (WBC) treatment on post exercise recovery.

Invitation and Purpose

You are being invited to participate in a research study examining the effects of WBC treatment timing on recovery from exercise. Taking part is voluntary and you have the right to physically withdraw at any point if you wish. Should you wish to take part, you will be asked to sign a consent form. WBC involves cold exposure to treat a variety of conditions. WBC is suggested to improve your recovery from exercise. WBC has also been shown to promote wellbeing, as well as having beneficial effects on quality of sleep.

Whole body cryogenic chamber at the Chris Moody Centre, which contains cold dry gas. This has previously been used by sports clubs such as Leicester Tigers and Northampton Saints.

What will happen to me if I take part and how long with my participation take?

Your whole participation will consist of 3 trials on separate days. Trial 1 is likely to take approximately 1.5 hours. Trial 2 is likely to take approximately 3 hours depending on timing of WBC treatment. Trial 3 is likely to take less than an hour. You will be asked to arrive at the University Waterside Campus at a designated date and time and to refrain from alcohol intake and strenuous activity for 24 and 48 hours before testing sessions.

You will be asked to complete a medical questionnaire. For your first trial, you will have several measures taken (see appendix for complete list of measurements). It is important that you meet the inclusion criteria and that you are free from conditions that could make exercise or the cryotherapy treatment unsafe. You will perform two exercise tests. The first involves treadmill running where you will have a face mask on and exercise at progressive intensities until exhaustion. The second will be an assessment of your leg muscle strength. You will be given a watch to monitor your sleep for 4 consecutive nights (3 nights prior to the main trial and the night following the cryotherapy treatment). You will also be given a sleep questionnaire to fill in for the same nights.

A few days after the initial testing, you will be asked to report to the University Waterside Campus for trial 2. You are also advised to avoid caffeine for at least 4 hours prior to this, but to ensure you are hydrated. Some measurements and blood samples (see appendix) will be taken before and after exercise. You will be asked to perform a treadmill downhill running session. Following the run,

further measurements will be taken and you will transport to the Chris Moody Centre (CMC) for the cryotherapy treatment. This treatment will occur either 1 hour or 4 hours following the treadmill run, depending on your allocation. If you are allocated to the 4 hour timing, you will be allowed to leave for 3 hours and instructions will be provided to arrive at CMC for your delayed cryotherapy treatment.The treatment involves exposure to cold dry gas for a duration of 3 minutes. You will be asked to move around inside the chamber and will be supervised throughout. If you are assigned as a control participant you will not undergo this cryotherapy treatment and will instead remain seated in the University Campus. The pre-treatment measures will be assessed on completion of the treatment (see trial 2 flow chart appendix).

The next day (trial 3), you will be asked to perform the same leg muscle strength test outlined above. Final blood samples will be collected. You will also be asked to submit the outcomes from the sleep assessments.

What are the possible disadvantages and risks of taking part?

The risks involved with exposure to cold air are mainly related to cold intolerance and discomfort. Steps will be taken to ensure your safety throughout, such as wearing specialised clothing to cover your extremities and you will be supervised the entire time. Other minor risks include small changes in blood pressure, cold allergic reactions and claustrophobia. Blood collection procedures may be associated with feeling faint or light-headed. You will be asked to assess your own thermal comfort and wellbeing levels regularly and it is important that you inform the researcher of any discomfort. As with any participation in strenuous exercise, it is usual to feel tired and sore afterwards. It is also important to reduce risk of injury by ensuring you are properly rested before exercise sessions.

What will happen to the data collected?

All data collected will be kept strictly confidential and stored safely on a password protected personal file in accordance with the General Data Protection Regulation (GDPR). Your research data will be kept anonymous and identified by initials. Once your data has been collected and analysed, they will be available for you to access at your request. The results will be shared among the project stakeholders and might be published in a journal. The individual findings will only be identified in reports or publications if you have provided your consent. Should you wish to withdraw, your data will not be used after 1 month following completion of your participation.

Further information and contact details:

If you have any concerns, queries or would like to make a complaint, you may contact the following:

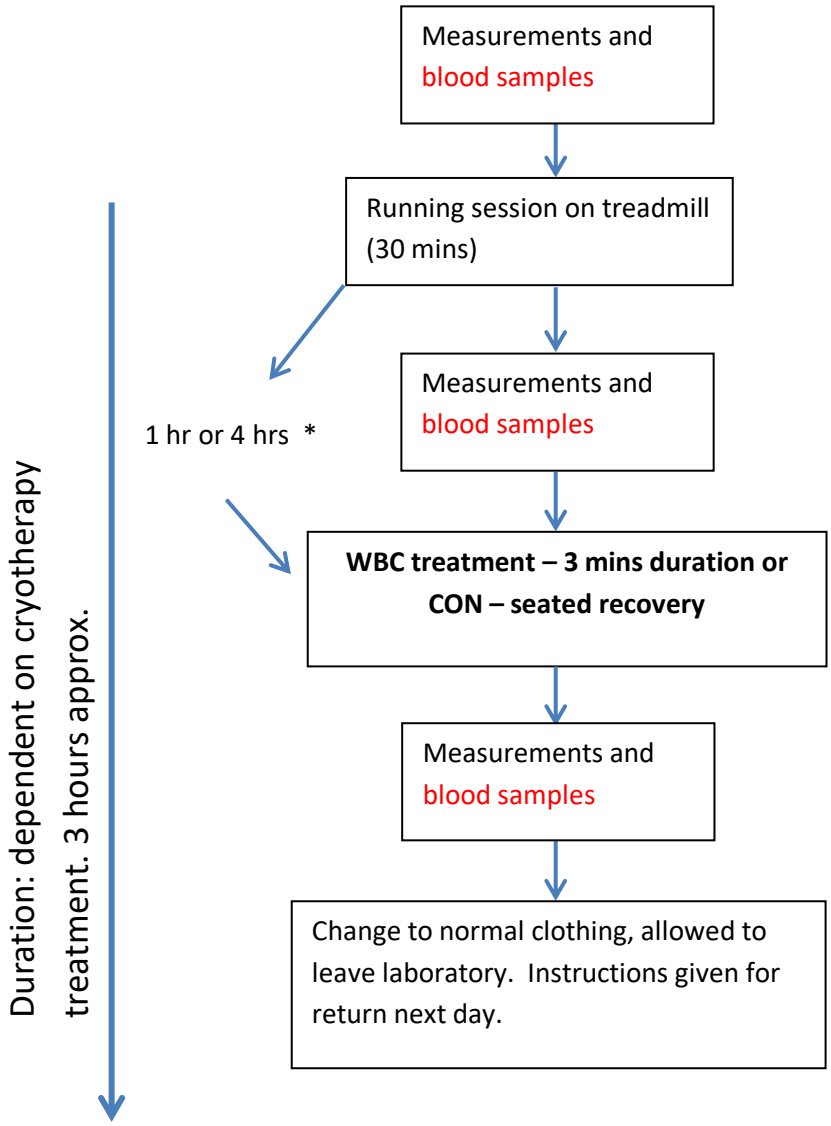
Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

Bill Ribbans, supervisory team, billribbs@uk-doctors.co.uk;

Chris Moody Sports Therapy and Injury Rehabiliation Centre - 01604 492 222, http://sportstherapy.moulton.ac.uk/Services/Cryotherapy

Appendix

Flow chart of procedures for trial 2 - Main experimental trial (Chris Moody Centre, a few days after preliminary visit):



WBC – Whole Body Cryotherapy

CON – Control group

* - 4 hour participants allowed to leave and instructed to return at designated time

The following measures will be taken:

Trial 1: Blood pressure, body fat (skinfold), mass and height, VO2 max, muscle torque,

Trial 2: Doppler ultrasound, blood samples (whole and fingerstick), tympanic temperature, skin temperature, muscle soreness, thermal comfort, wellbeing.

Trial 3: Blood samples (whole and fingerstick), muscle soreness, wellbeing, muscle torque.

Sleep measured by watch and questionnaire for 3 nights prior to trial 2 and the night following trial 2.

The nature and timings of these measures will be explained in more detail in person.

Title of Study

The impact of repetitive Whole Body Cryotherapy (WBC) treatment on adaptations to exercise training.

Invitation and Purpose

You are being invited to participate in a research study examining the effect of repetitive WBC treatment on long term adaptations to exercise training. Taking part is voluntary and you have the right to physically withdraw at any point if you wish. Should you wish to take part, you will be asked to sign a consent form. WBC involves cold exposure to treat a variety of conditions. WBC is suggested to improve your recovery from exercise. WBC has also been shown to promote wellbeing, as well as having beneficial effects on quality of sleep.

Whole body cryogenic chamber at the Chris Moody Centre, which contains cold dry gas. This has previously been used by sports clubs such as Leicester Tigers and Northampton Saints.

What will happen to me if I take part and how long with my participation take?

Your whole participation will involve a training programme lasting 6 weeks, with exercise performance tests before and after the programme. Each training week, you will perform 4 exercise sessions on alternating days – 2 running sessions on a treadmill and 2 strength training sessions (e.g. weights). Each training session will last less than an hour. You will be asked to arrive at the University Waterside Campus or Moulton College at designated dates and times and to refrain from strenuous activity and alcohol intake for 24 hours before sessions.

You will be asked to complete a medical questionnaire and have several measures taken (e.g. ultrasound for leg blood flow). It is important that you meet the inclusion criteria and that you are free from conditions that could make the cryotherapy treatment unsafe. You will be explained all testing procedures to follow.

There will be four exercise performance tests. Your aerobic fitness will be assessed by running on a treadmill where you will have a face mask on and exercise at progressive intensities until exhaustion. Your muscle strength will be assessed by specialised equipment and the weight lifted for a barbell squat (see diagrams below). Your power will be assessed by a jump test. You will also have your body composition assessed.

A few days after the initial testing, you will commence the 6 week training programme, training 4 times each week. You will undergo two cryotherapy treatments each week, occurring after two of the training sessions. The treatment involves exposure to cold dry gas for a total duration of 3 minutes. You will be asked to move around inside the chamber and will be supervised throughout. After completing the 6 week programme, you will be asked to perform the exercise performance tests again (as referred to above).

What are the possible disadvantages and risks of taking part?

The risks involved with exposure to cold air are mainly related to cold intolerance and discomfort. Steps will be taken to ensure your safety throughout, such as wearing specialised clothing to cover your extremities and you will be supervised the entire time. Other minor risks include small changes in blood pressure, cold allergic reactions and claustrophobia. It is important that you inform the researcher of any discomfort. As with any participation in strenuous exercise, it is usual to feel tired and sore afterwards. It is also important to reduce risk of injury by ensuring you are properly rested.

What will happen to the data collected?

All data collected will be kept strictly confidential and stored safely on a password protected personal file in accordance with the General Data Protection Regulation (GDPR). Your research data will be kept anonymous and identified by initials. Once your data has been collected and analysed, they will be available for you to access at your request. The results will be shared among the project stakeholders and might be published in a journal. The individual findings will only be identified in reports or publications if you have provided your consent. Should you wish to withdraw, your data will not be used after 1 month following completion of your participation.

Further information and contact details:

If you have any concerns, queries or would like to make a complaint, you may contact the following:

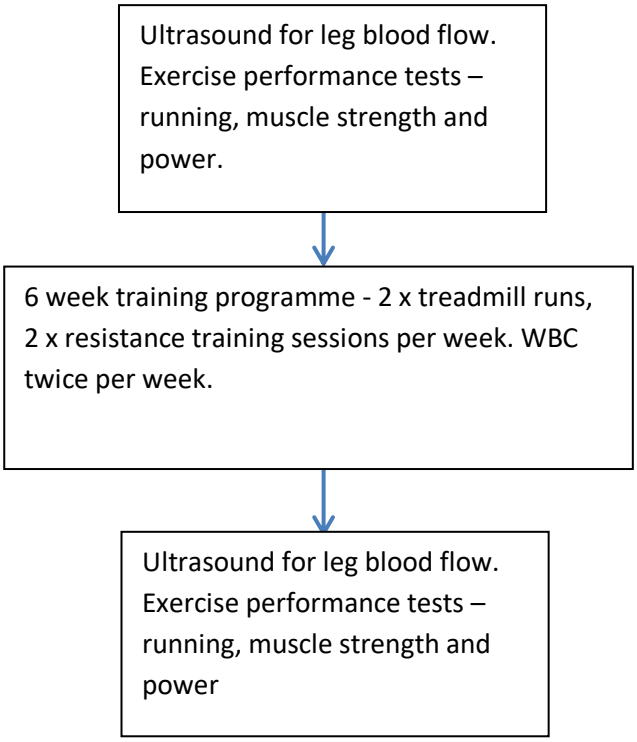
Adnan Haq, principal investigator – 07791545203, adnan.haq@moulton.ac.uk

Moulton College, Moulton, Northamptonshire, NN3 7RR

Chris Moody Sports Therapy and Injury Rehabiliation Centre - 01604 492 222, http://sportstherapy.moulton.ac.uk/Services/Cryotherapy

Appendix

Flow chart of procedures for study participation – 6 week training programme and performance tests.



WBC – Whole Body Cryotherapy

Programme of Exercises for Training Study

Strength + Plyometric training – 2x/week:

Barbell squats – 4 sets of 6 (70% estimated 1RM => progressing to 75% after 3 weeks)

Dumbbell lunges – 3 sets of 8

Nordic leg curls – 2 sets of 8

Depth jumps – 3 sets of 8

Split lunge jumps – 3 sets of 8

2 mins recoveries between sets

Running – 2x/week: 30 minutes treadmill at 70% VO_2 max (progressing to 75% after 3 weeks)

Sessions arranged throughout week in sequence run=>strength=>run=>strength (exact days subject to participant availability).

Pre and post tests for ultrasound, body fat, VO_2 max, 3RM squat, muscle torque, vertical jump power

PhD – Whole Body Cryotherapy and Sports Recovery

Participant Data Sheet for Pilot Study 1

Name: DOB:

Date:

Pre-test measures

Body mass: Height:

Blood pressure (x3):

Dynamometer dimensions: Back rest - Chair -

Baseline Muscle Torque Assessment 1 (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Baseline Muscle Torque Assessment 2 (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Baseline Muscle Torque Assessment 3 (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

EIMD Exercise Protocol

Estimated max HR (202 - (0.55 x age): 70% of max HR:

Running Duration (mins)	Running speed (km/h)	Heart Rate (bpm)	Rating of Perceived Exertion (6-20 Borg scale)
0			
2			
4			
5			
10			
15			
20			

Post EIMD Muscle Torque Assessment (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Trial 2 – Baseline Muscle Torque Assessment (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

EIMD Exercise Protocol

Estimated max HR (202 - (0.55 x age): 70% of max HR:

Running Duration (mins)	Running speed (km/h)	Heart Rate (bpm)	Rating of Perceived Exertion (6-20 Borg scale)
0			
2			
4			
5			
10			
15			
20			

Post EIMD Muscle Torque Assessment (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

PhD – Whole Body Cryotherapy and Sports Recovery

Participant Data Sheet for Pilot Study 3

Name: DOB:

Date:

Pre-test measures

Body mass: Height: Blood pressure (x3):

Dynamometer dimensions: Back rest - Chair length - Chair height –

Lever arm length –

One rep max tests:

Barbell Squat - Dumbbell Lunge - Leg extension -

70% of one rep max:

Barbell Squat - Dumbbell Lunge - Leg extension -

EIMD randomisation: Downhill run => Resistance Resistance => Downhill run

Muscle Torque Assessment 1, pre EIMD (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Muscle Torque Assessment 2, post EIMD (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Muscle Torque Assessment 3, pre EIMD (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Muscle Torque Assessment 4, post EIMD (Muscle soreness -)

Contraction 1	Contraction 2	Contraction 3	Contraction 4	Peak Torque

Downhill Running Protocol

Estimated max HR (202 - (0.55 x age): 70% of max HR:

Running Duration (mins)	Running speed (km/h)	Heart Rate (bpm)	Rating of Perceived Exertion (6-20 Borg scale)
0			
2			
4			
5			
10			
15			
20			

Data Sheet for Main Study 1

Name DOB Date

Pre test Measures

Height Body mass Blood Pressure x 3

Skinfolds (inc. means): Bicep - Tricep -

Subscapular - Iliac crest - TOTAL -

Estimated body fat% -

VO2 max (see separate sheet for more details) -

Muscle torque (inc. familiarisation)

Dynamometer dimensions: Back rest - Chair length + height - Lever arm length -

1. 2. 3. 4. Peak torque:

Main Trial Ambient temp -

Tympanic temp - Skin temp – Blood CK - Blood Hb –

Muscle soreness – VAS thermal comfort – VAS wellbeing –

Downhill run – 30 mins at 60% VO2 max. Resting HR -

Target HR at 60% VO_2 max =

Running Duration (mins)	Running speed (km/h)	Heart Rate (bpm)	Rating of Perceived Exertion (6-20 Borg scale)
0			
2			
5			
10			
15			
20			
25			
30			

(Immediately post downhill run):

Tympanic temp - Skin temp – Blood CK - Blood Hb –

Muscle soreness – VAS thermal comfort – VAS wellbeing –

WHOLE BODY CRYOTHERAPY TREATMENT AT CMC 60 mins post downhill run (if applicable)

Ambient temp -

(Post WBC measures, 10 mins post except skin temp): HR -

Tympanic temp - Skin temp (5 mins post WBC) – Blood CK - Blood Hb –

Muscle soreness – VAS thermal comfort – VAS wellbeing –

Trial 3 - 24 hours post downhill run

Muscle torque –

1. 2. 3. 4. Peak torque:

Blood CK - Blood Hb - Muscle soreness –

VAS wellbeing –

Skin temperatures (post FLIR tools analysis)

Measure 1 (pre): Chest - Tricep - Thigh - Calf – Mean -

Measure 2 (post downhill): Chest - Tricep - Thigh - Calf – Mean -

Measure 3 (post WBC): Chest - Tricep - Thigh - Calf – Mean

Data Sheet for Main Study 2

Name DOB Date

Pre test Measures

Height Body mass Blood Pressure x 3

Skinfolds (inc. means): Bicep - Tricep -

Subscapular - Iliac crest - TOTAL -

Estimated body fat% - VO2 max (see separate sheet for more details) -

Muscle torque (inc. familiarisation)

Dynamometer dimensions: Back rest - Chair length + height - Lever arm length -

1. 2. 3. 4. Peak torque:

Main Trial Ambient temp -

Tympanic temp - Skin temp – Blood CK - Blood Hb –

Muscle soreness – VAS thermal comfort – VAS wellbeing –

Downhill run – 30 mins at 60% VO2 max. Resting HR -

Target HR at 60% VO_2 max =

Running Duration (mins)	Running speed (km/h)	Heart Rate (bpm)	Rating of Perceived Exertion (6-20 Borg scale)
0			
2			
5			
10			
15			
20			
25			
30			

(Immediately post downhill run):

Tympanic temp - Skin temp – Blood CK - Blood Hb –

Muscle soreness – VAS thermal comfort – VAS wellbeing –

WHOLE BODY CRYOTHERAPY TREATMENT AT CMC 60 mins/4hrs post downhill run (if applicable)

Time of day of cryotherapy treatment -

(Post WBC measures, 10 mins post except skin temp): HR -

Tympanic temp - Skin temp (5 mins post WBC) – Blood CK - Blood Hb –

Muscle soreness – VAS thermal comfort – VAS wellbeing –

Trial 3 - 24 hours post downhill run

Muscle torque –

1. 2. 3. 4. Peak torque:

Blood CK - Blood Hb - Muscle soreness –

VAS wellbeing –

Skin temperatures (post FLIR tools analysis)

Measure 1 (pre): Chest - Tricep - Thigh - Calf – Mean -

Measure 2 (post downhill): Chest - Tricep - Thigh - Calf – Mean -

Measure 3 (post WBC): Chest - Tricep - Thigh - Calf – Mean

Sleep Assessments (FitBit)

3 nights before WBC -

2 nights before WBC -

Night before WBC -

Night following WBC -

Data Sheet for Training Study

Name DOB Date

Pre test Measures

Height Body mass Blood Pressure

Skinfolds (inc. means): Bicep - Tricep -

Subscapular - Iliac crest - TOTAL -

Estimated body fat% - VO2 max (see separate sheet for more details) -

Target HR at 70% VO_2 max =

Target HR at 75% VO_2 max =

Muscle torque (inc. familiarisation)

Dynamometer dimensions: Back rest - Chair length + height - Lever arm length -

1. 2. 3. Peak torque:

3RM squat –

Estimated 1RM – 70% 1RM - 75% 1RM -

CMJ Power –

Doppler ultrasound:

Velocity –

Diameter -

Post test Measures

Height Body mass

Skinfolds (inc. means): Bicep - Tricep -

Subscapular - Iliac crest - TOTAL -

Estimated body fat% - VO2 max (see separate sheet for more details) -

Muscle torque (inc. familiarisation)

Dynamometer dimensions: Back rest - Chair length + height - Lever arm length -

1. 2. 3. Peak torque:

3RM squat –

Estimated 1RM –

CMJ Power –

Doppler ultrasound:

Velocity –

Diameter -

VO_2 max test sheet

Participant name

DOB Date of Test

Height Body mass

Stage	Speed (km/h)	Gradient (%)	VO_2	HR	RPE
1 (0-2 mins)	6	1			
2 (2-4 mins)	8	1			
3 (4-6 mins)	10	1			
4 (6-8 mins)	12	1			
5 (8-10 mins)	14	1			
6 (10-12 mins)	16	1			
7 (12-14 mins)	16	3			
8 (14-16 mins)	16	5			
9 (16-18 mins)	16	7			

VO_2 max =

60% VO_2 max =

Corresponding HR at 60% VO_2 max =

70% VO2 max =

Corresponding HR at 70% =

75% VO2 max =

Corresponding HR at 75% =

University of Northampton

COVID-19 – Questionnaire for Research Participants

Name…… Date……..

Please complete the following questions accurately and honestly, highlighting YES or NO where necessary.

Have you tested positive for COVID-19 at any point? YES NO

If yes, please specify when.

Have you experienced any of the following symptoms in the past 14 days?

Fever (temperature consistently above 38°C) YES NO

Dry and persistent cough YES NO

Loss of smell or taste YES NO

Shortness of breath/difficult breathing during rest YES NO

Muscle pain during rest (not including exercise related soreness) YES NO

Headaches YES NO

If YES to any of the above symptoms, please provide further details:

Have you been identified as a close contact of someone who has tested positive for COVID-19? YES NO

If so, please provide details.

If you have any queries, please contact the researcher Adnan Haq on adnan.haq@moulton.ac.uk or 07791545203.

University of Northampton

COVID-19 – Instructions for Research Participants

by Adnan Haq

PLEASE READ CAREFULLY

You are aware of the current situation regarding COVID-19 and the impact it has had. Clearly, for any laboratory based research work to advance, certain guidelines need to be met to reduce risk of transmission as far as possible, both by the researcher and participants. Please be advised that in order to do so, all participants will be treated as if they are infected.

It is therefore essential that you are aware of all guidelines before signing your consent to take part in this research. Please ensure you read the following guidelines regarding risk mitigation of COVID-19 during participation in my study:

- All forms are to be completed and sent electronically. Where necessary you will be required to fill in scales electronically (e.g. muscle soreness scales).

- Please ensure you bring your own fluids and towel/paper towels to lab sessions. You may wish to wear your own facemask where possible.

- Please ensure you are **wearing clean clothes** upon starting each lab session. This may mean changing clothes if you are coming straight from work. You may prefer to bring spare clothing anyway. Participants are not permitted to use changing facilities. Otherwise, please try to avoid bringing excess clutter in order to assist keeping the lab spaces clean and clear.

- Please ensure you **wash your hands** thoroughly upon laboratory entry and at regular intervals during sessions. There will also be several hand gels available.

- **Social distancing** (2 metres+) shall be adhered to in the laboratory. There may be other researchers/participants present in the lab at the same time. When social distancing is not possible (e.g. direct body contact during certain exercise tests, blood samples etc.), the researcher will apply protective measures.

- You will be asked to apply certain measures yourself without researcher intervention where possible (e.g. monitoring heart rate, body mass)

- You will be asked to **monitor and report any symptoms specific to COVID-19** for 14 days prior to study participation and throughout the study. Any positive test must also be reported. You will also be asked to inform the researcher if you are identified as a close contact of someone tested positive for COVID-19. Whilst it is understood that it is not always practical, you will be asked to minimise close contact with others where possible throughout your participation.

- Unless absolutely necessary, please try to avoid using other facilities in the University campus outside of the sports science labs (e.g. changing rooms, showers).

Please inform the researcher if you experience any of the following symptoms:

- Fever (temperature consistently above 38°C)
- Dry and persistent cough
- Loss of smell or taste
- Shortness of breath/difficulty breathing during rest
- Muscle pain during rest (does not include exercise related soreness)

As per government guidelines, you are advised to have a test for COVID-19 should you present any of the symptoms.

Please sign below to confirm that you have read and understood all instructions:

Name…….

Signature……. Date…….....

If you have any queries, please contact the researcher Adnan Haq on adnan.haq@moulton.ac.uk or 07791545203.

University of Northampton

Moulton COLLEGE

Research Participants Needed!

Volunteers are required to participate in a study examining the effects of Whole Body Cryotherapy (WBC) treatment for sports recovery. WBC involves safe and brief exposure to extreme cold with some reported effects including reduced pain/ soreness, better recovery, mobility, wellbeing and sleep. Are you:

- Interested in sport and/or exercise?
- Interested in how sports recovery can be improved?
- Keen to try out new methods to promote recovery from sport?

If you are a reasonably fit and healthy male aged 18 or over, then why not take part in research that aims to assess the impact of this novel tool for sports recovery and ***receive potentially over £100 worth of sports science treatment?***

If you are interested, please contact **Adnan Haq** for further details.

07791545203
adnan.haq@moulton.ac.uk

Moulton College, West Street,
Moulton, Northamptonshire,
NN3 7RR

University of Northampton

Moulton COLLEGE

Research Participants Needed for Sports Science Study!

Volunteers are required to participate in a study examining the effects of Cold Water Immersion (CWI) treatment for sports recovery. CWI involves brief immersion (10 mins) in cold water with some reported effects including reduced pain/soreness, reduced swelling, better recovery and performance. Are you:

- Interested in sport and/or exercise?
- Interested in how sports recovery can be improved?
- Keen to try out different methods to promote recovery from sport?

If you are a reasonably fit and healthy male aged 18 or over, then why not take part in research that aims to assess the impact of this common tool for sports recovery and ***receive over £50 worth of sports science treatment?***

If you are interested, please contact

Adnan Haq for further details.

07791545203
adnan.haq@moulton.ac.uk

Moulton College, West Street,
Moulton, Northamptonshire,
NN3 7RR

University of Northampton

Moulton COLLEGE

Research Participants Needed for Cryotherapy Training Study!

Volunteers are required to participate in a study examining the effects of frequent Whole Body Cryotherapy (WBC) treatment on responses to a 6 week exercise training programme. WBC involves safe and brief exposure to extreme cold with some reported effects including reduced pain/soreness, better recovery, mobility, wellbeing and sleep. Are you:

- Interested in sport and/or exercise?
- Keen to improve your strength and fitness?
- Keen to try out new methods to promote sports recovery?

If you are a healthy male aged 18 or over, then why not take part in research that aims to assess the impact of this novel tool for sports training and ***receive potentially over £600 worth of sports science treatment?***

If you are interested, please contact

Adnan Haq for further details.

07791545203
adnan.haq@moulton.ac.uk

Moulton College, West Street,
Moulton, Northamptonshire,
NN3 7RR