Effects of cold-water immersion on post-exercise recovery of skeletal muscle function following acute sprint-interval exercise

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN KINESIOLOGY AND HEALTH SCIENCE

YORK UNIVERSITY

TORONTO, ONTARIO

September 2021

Abstract

Cold-water immersion (CWI) is a popular post-exercise intervention, assumed to be effective in accelerating muscle recovery after strenuous exercise. Previous evidence has proposed that acute increases in oxidative stress induced by SIE cause a long-lasting depression in submaximal force generation called prolonged low-frequency force depression (PLFFD). The focus of this thesis was to test the hypothesis as to whether CWI can enhance recovery of neuromuscular function by decreasing oxidative stress and mitigating the extent of PLFFD. PLFFD was examined by measuring torque from evoked stimulation at low- and high-frequencies before, immediately after and 24h post-exercise. Results showed that PLFFD was observed at 24h post-exercise but no difference was seen in the extent of PLFFD between the cooled and control leg. These results do not support any benefit of acute 30 min post-exercise CWI on the recovery of skeletal muscle function at 24h following SIE.

Acknowledgments

I would like to thank my supervisor Dr. Arthur J. Cheng for his guidance throughout the course of these 2 years. Despite the circumstances of the COVID-19 Pandemic, you have continued to provide me with constant support and helped me learn from the many mistakes I made. Your patience with me and your passion within your field gave me the drive to complete this project with determination. There were moments where I thought to myself that I might need an extra year or two to finish this project, but you gave me the motivation needed to understand and learn about my project. Without your help, I wouldn't have been able to look at the bigger picture and ask myself why I did what I did- and for that I thank you tremendously.

I also want to thank Dr. Geoffrey Power and his lab members at the University of Guelph for taking the time to teach me all the techniques and concepts needed in this project. The one week that I stayed at Guelph allowed me to obtain all the data I presented in my thesis. The pandemic ruined future plans for this project, but I am truly grateful for Dr. Geoffrey Power and his lab members' hospitality and help.

Lastly, I would like to send out a huge thank you to Dr. Thomas Chaillou at Örebro University and Viktorja Treigyte at Lithuanian Sports University for helping me develop my thesis and understand the background literature for cold-water immersion studies. Additionally, I am grateful for both of them for considering me as a contributor for a literature review that is soon to be published. Our monthly zoom meetings highly contributed to my knowledge of all the literature mentioned in my thesis and they were extremely helpful.

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List of Abbreviations

$[Ca^{2+}]_i$: Myoplasmic free calcium concentration

ATP: Adenosine 5'-triphosphate

BMI: Body mass index

Ca^{2+}: Calcium

CMJ: Countermovement jump

CWI: Cold- water immersion

EE: Endurance exercise

H^+: Hydrogen ion

HIIE: High-intensity interval exercise

CON: Control condition

HSP: Heat shock protein

Hz: Hertz

M_{max}: Maximum m-wave amplitude

MRR: Maximal rate of torque relaxation

MRTD: Maximal rate of torque development

MVC: Maximal voluntary contractions

MVIC: Maximal voluntary isometric contractions

NADPH: Nicotinamide adenine dinucleotide phosphate

N·m: Newton·metres

PGC-1α: Peroxisome proliferator-activated receptor-gamma coactivator

P_i: Inorganic phosphate

PLFFD: Prolonged low-frequency force depression

POST: Immediately after task failure

PRE: Baseline, unfatigued state

RONS: Reactive oxygen and nitrogen species

ROS: Reactive oxygen species

RyR: Ryanodine receptor

SIE: Sprint-interval exercise

SIT: Sprint-interval training

SR: Sarcoplasmic reticulum

TMS: Transcranial magnetic stimulation

TWI: Thermoneutral-water immersion

VO_{2max}: Maximal rate of oxygen consumption, maximal aerobic capacity

CHAPTER 1: INTRODUCTION

Sprint interval exercise (SIE) is a sub-form of high intensity interval exercise (HIIE), involving repeated bouts of brief <1 min "all-out" exercise, performed with short rest periods between each bout (Wingo et al., 2018). SIE is also relevant to various sports (e.g., soccer, ice hockey, basketball), whereby sporting events can involve bursts of maximal effort interspersed with periods of rest (Wingo et al., 2018). Due to the strenuous nature and high metabolic demand of SIE, this type of acute exercise leads to fatigue-induced reductions in muscle contractile force and power following SIE (Leveritt et al., 1999; Place et al., 2015; Fernandez-del- Olmo et al., 2013). During voluntary exercise, fatigue can be attributed to impairments in the neuromuscular system and the factors contributing to fatigue are typically distinguished as central and peripheral fatigue; central fatigue is identified as neural contributions to the decrease in voluntary activation of skeletal muscle while peripheral fatigue is exhibited as a decrease in skeletal muscle force or power generation due to intramuscular mechanisms (Mira et al., 2018). It is well-known that depending on the exercise task performed, the contributions of central and peripheral factors to exercise-induced fatigue will differ (Allen et al., 2008; Gandevia et al., 2001) However, due to the increasing popularity of SIE as a training paradigm, only recently have studies investigated the neuromuscular factors contributing to fatigue during SIE.

The few studies that have examined the neuromuscular contributions to fatigue during SIE have shown evidence of both central and peripheral origins of fatigue (Fernandez-del-Olmo et al., 2013; Place et al., 2015). Fernandez-del-Olmo et al. (2013) showed that short, 30 s Wingate bouts of supramaximal cycling were associated with a significant decrease of maximal voluntary isometric contraction (MVC) force following an isometric knee extension force test.

Specifically, MVC force in the knee extensors declined by ~16% after each 30s Wingate while voluntary activation, assessed by twitch interpolation, also decreased significantly by ~ 27% between the 1st and 2nd set of each Wingate (Fernandez- del- Olmo et al., 2013). Thus, the fatigue-induced reduction in voluntary force has been attributed to central fatigue. Furthermore, additional evidence showed that SIE causes a fatigue-induced depression in electrically evoked force in human and mouse skeletal muscle (Place et al., 2015), indicating an intramuscular impairment in muscle force generation. In summary, these results indicate that SIE may be associated with both central and peripheral fatigue.

Intense exercise such as SIE also results in a long-lasting fatigue in the post-exercise recovery period which is typically exhibited as a depression (>24h) in submaximal force generation called prolonged low-frequency force depression (PLFFD) (Place et al., 2015; Skurvydas et al., 2017). PLFFD is observed as a greater depression in electrically evoked force at submaximal compared with maximal stimulation frequencies (Allen et al., 2008), and can be reflected as a ratio of low-to-high frequency force (Cheng et al., 2020; Martin et al., 2004; Watanabe et al., 2019). It was recently shown that the intramuscular mechanisms underlying PLFFD following SIE are decreased SR Ca^{2+} release and decreased myofibrillar Ca^{2+} sensitivity, which cause a rightward shift in the force-$[Ca^{2+}]_i$ relationship such that the observed force deficit at low stimulation frequencies is greater than that observed at high stimulation frequencies (Cheng et al., 2020) (Figure 1). In other words, submaximal contractions are a more sensitive measure of persisting fatigue during the post-exercise recovery period than maximal contractions, consequently indicating PLFFD. Furthermore, PLFFD has a functional relevance since most exercise tasks involve submaximal contractions, and the presence of PLFFD indicates that muscle weakness persists long after the exercise has been completed. PLFFD will likely

increase the perceived voluntary effort required for any exercise task since increased neural activation will be required to overcome the muscle weakness caused by persisting PLFFD in the post-exercise recovery period. Thus, any intervention that can be utilized to improve recovery by reversing PLFFD may be beneficial for improving athletic performance.

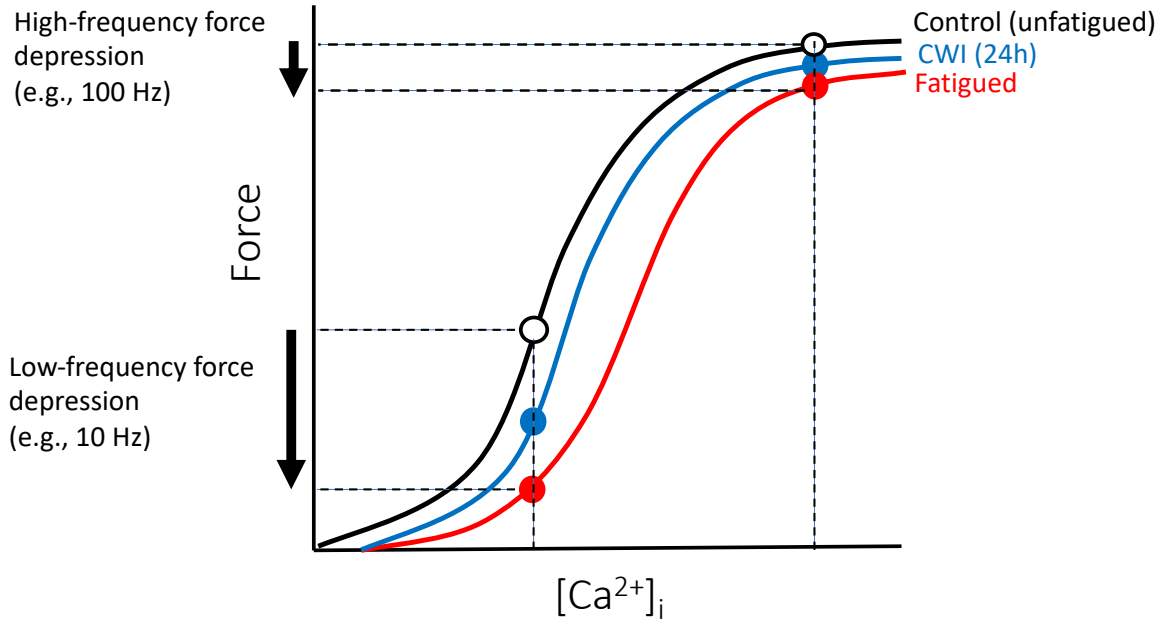

Figure 1. Force-calcium relationship and prolonged low-frequency force depression Conceptual illustration of the sigmoidal force-calcium relationship showing a greater fatigue-induced depression in electrically evoked at low (10 Hz) vs. high (100 Hz) frequencies assessed at the same timepoint, signifying prolonged low-frequency force depression (PLFFD). The black line represents the force-calcium relationship in unfatigued skeletal muscle. The blue line represents the force-calcium relationship in skeletal muscle at 24h post-exercise recovery following CWI. The red line represents the force-calcium relationship in fatigued skeletal muscle without CWI treatment. $[Ca^{2+}]_i$ refers to myoplasmic free calcium concentration.

There has been great interest to determine effective post-exercise recovery interventions to accelerate the recovery of skeletal muscle contractile function following fatigue. Accelerating recovery from fatigue is relevant for athletes who train for competitive sports that occur on

consecutive days whereby improved management of fatigue symptoms and prevention of fatigue accumulation may dictate athletic performance in multi-day competitions. Muscle force generation is an important determinant of exercise performance and quantifying the extent to which force recovers following fatigue-induction can provide an accurate evaluation of recovery effectiveness following fatigue.

To that end, an increasingly common and popular post-exercise modality used amongst both athletes and non- athletes is cold water immersion (CWI), with the assumption that CWI is effective in promoting muscle recovery. CWI is a post-exercise regimen, involving the submersion of a specific limb or the whole body in cold-water with temperatures < 15°C, such as an ice bath (Bleakley et al., 2012). The effectiveness of CWI as a recovery intervention has been assessed following resistance exercise and eccentric contractions that induce muscle damage (Fröhlich et al., 2014; Pointon et al., 2011; Wilson et al., 2018) as well as following prolonged endurance exercise (Peiffer et al., 2010; Vaile et al., 2008a; De Paula et al., 2018). Acute CWI is generally thought to be beneficial based on the presumption that CWI decreases muscle soreness, stimulates vasoconstriction and reduces intramuscular swelling following exercise (Hyldahl et al., 2020). Nonetheless, some of the literature from resistance and endurance exercise has not supported any beneficial effect of CWI during the later recovery phase (24-72h), and if anything, possible detrimental effects of CWI as a recovery intervention for improving strength (Cheng et al., 2017; Hyldahl et al., 2020). This may be due to a lack of sufficient intramuscular cooling to affect recovery processes, or in the instances of severe cooling, there could be vasoconstriction in limbs and reduced blood flow to skeletal muscles, as well as slowed metabolic processes that impairs glycogen resynthesis and muscle protein synthesis (Cheng et al., 2017; De Paula et al., 2018; Fuchs et al., 2020; Stanley et al., 2012; Hyldahl et al., 2020).

Nevertheless, the proposed mechanisms by which CWI may affect recovery of muscle function following SIE may differ from those of resistance and eccentric exercise since SIE causes minimal muscle damage as shown by modest elevations in plasma creatine kinase (Bogdanis et al., 2013). Furthermore, SIE is also not associated with substantial glycogen depletion (Gollnick et al., 1973), which on the other hand is a major cause of fatigue during prolonged endurance exercise (Cheng et al., 2017b; Ørtenblad et al., 2013). The unique feature of SIE is that this high-intensity type exercise induces substantial oxidative stress in skeletal muscle due to the intramuscular generation of reactive oxygen species (ROS) (Sakellariou et al., 2013). Similarly, the development of PLFFD following SIE is also known to be reactive oxygen and nitrogen species (RONS)-dependent (Cheng et al., 2015; Place et al., 2015; Watanabe et al., 2019). It is known that lowering intramuscular temperature can reduce ROS generation in isolated rodent muscle (Poel et al., 2008), and thus it could be hypothesized that CWI applied at the human in-vivo level could effectively lower oxidative stress in the post-exercise recovery period.

In the following section, I will review the existing evidence on the efficacy of CWI as a post-exercise recovery intervention on neuromuscular function. Although CWI has been used as a recovery intervention following resistance, endurance, and sprint-type exercises, the following literature review will focus predominantly on the more closely related exercise types: endurance and sprint-type exercises. This will then lead to my hypothesis that CWI will mitigate the recovery of neuromuscular function following acute SIE.

CHAPTER 2: LITERATURE REVIEW

2.1: Introduction to Cold Water Immersion as a Recovery Modality

2.1.1 Overview of Cooling and Exercise

Cold water immersion (CWI) techniques have become a common post-exercise intervention adopted by athletes based on the assumption that CWI accelerates the recovery of skeletal muscle function after strenuous exercise (Hydahl et al., 2020). Various methods of cooling exist, such as cryotherapy and local icing, however CWI is typically defined as immersion in cold temperatures of ≤ 15-20 °C (White et al., 2014; Wingo et al., 2018; Hyldahl et al., 2020). It has been widely popular to apply CWI in the post-exercise recovery period following endurance-type exercise given that endurance sporting events can involve multi-day (e.g., World Championships, Olympics, elimination-round playoff events) and multi-week competitions (e.g., Tour de France) where CWI is assumed to play a role in accelerating acute recovery between competition days. The mechanisms by which CWI may affect acute post-exercise recovery processes remain poorly understood (Hyldahl et al., 2020), but attempts have been made to determine the effectiveness of CWI as a recovery modality at the whole-body level by assessing functional outcome measures of endurance tests or assessments of strength and power. These functional outcome measures are critical as they serve as the foundation to determine whether CWI ultimately affects human exercise performance. Some evidence indicates that CWI performed after consecutive days of endurance exercise may have a positive effect on skeletal muscle fatigue resistance (Crampton et al., 2013; Vaile et al., 2008a). These positive effects on the recovery of neuromuscular performance include lower perceived muscle soreness, strength recovery, lower increase in creatine kinase (Ascenaso et al., 2011) as well as

improvements in the performance of counter movement jumps (CMJ), recover y of isometric strength and power (Vaile et al., 2008; Ascenaso et al., 2011; Sánchez-Ureña et al., 2018). However, other studies have shown that post-exercise CWI seems to have a negative effect on the recovery of neuromuscular function (i.e., strength, power) following endurance exercise. Evidence has shown that post-exercise CWI impaired the acute recovery of muscle strength (measured as maximum voluntary isometric contraction (MVIC) torque) and countermovement jump performance (CMJ) when cooling was executed immediately following endurance exercise (Peiffer et al. 2009a). Less is known about the effects of post-exercise CWI following sprint interval exercise (SIE) on the recovery of neuromuscular function. SIE is relevant to some sporting events involving repeated brief sprint efforts (e.g., soccer, ice hockey, basketball) and this form of exercise is also becoming a highly common time-efficient training method amongst athletes; however, such exercise is known to induce substantial fatigue-induced reductions in strength following SIE (Place et al., 2005; Christiansen et al., 2018). The mechanisms underlying fatigue from prolonged endurance exercise vs. high-intensity-type exercise (i.e., SIE) are known to be different as are the mechanisms involved in the post-exercise recovery processes (Cheng et al., 2016: Cheng et al., 2017; Cheng et al., 2020). Thus, the effectiveness of CWI as a recovery intervention may be exercise-type dependent.

The following literature review will examine the existing evidence on the influence of post-exercise CWI on the acute recovery of fatigue resistance and neuromuscular function following endurance exercise as well as following SIE, where it will be possible to compare the potential efficacy of CWI as a recovery modality following these two exercise types. The following sections will also be organized according to the timepoint of the functional outcome

measures being assessed in the acute post-exercise recovery period, progressing from 5 min after CWI application and up to 72h post-exercise.

2.1.2 Effects of local cooling on skeletal muscle recovery following endurance exercise

Endurance exercise is prolonged exercise that uses the cardiorespiratory system and is linked to improved capacity for aerobic energy (Egan et al., 2013). Additionally, endurance exercise is typically performed at intensities below maximal oxygen uptake (VO_{2max}) (Crampton et al., 2013). Below, I will present the evidence on the effects of acute CWI following endurance exercise at different recovery timepoints. For a summary of these studies, refer to Figure 2 and Table 1.

2.1.3 Effects of post-endurance exercise cooling on recovery at 5-30 min following exercise

Evidence shows that acute CWI (i.e., 5-20°C for 5-30 min) following endurance exercise improves the recovery of fatigue resistance (Crampton et al. 2013; Dunne et al. 2013; McCarthy et al. 2016; Peiffer et al. 2010; Vaile et al. 2008a; Yeargin et al. 2006). The study by Crampton et al., 2013 showed that time to failure during endurance cycling at 80% VO_{2max} was longer in the CWI treated group (15°C for 30 min) vs. the control groups (i.e., active recovery, contrast therapy or thermoneutral water immersion) when measured immediately following the 30 min CWI intervention. The potential mechanism by which repeated bout fatigue resistance improves immediately after acute CWI appears to be through the thermoregulatory action whereby systemic rather than local cooling per se with CWI is thought to enhance blood flow redistribution to active muscle, reduce the perceived effort and limit central fatigue (Ihsan et al. 2016).

2.1.4 Effect of post-endurance exercise cooling on recovery at 1-2h following exercise

On the other hand, these results contrast with those from prolonged 2 h of local muscle cooling, which had a negative effect on the recovery of fatigue resistance after exercise involving 60 min of moderate-intensity arm cycling in humans (Cheng et al., 2017b). In the latter study, the impaired recovery of fatigue resistance was attributed to reduced muscle glycogen resynthesis, with glycogen being a major cause of muscle fatigue during endurance exercise. To examine the mechanisms related to temperature-dependent recovery of muscle strength in the 2h post-exercise recovery period, a study by Cheng et al. performed ex-vivo experiments with mouse single intact muscle fibers that were fatigued with an endurance "exercise" stimulation protocol followed by a prolonged 2h recovery at 16°C, 26°C, 31°C, and 36°C. Compared with the control temperature (31°C), prolonged cooling (lower temperatures: 16°C and 26 °C) impaired the recovery of submaximal (30 Hz) evoked force. The mechanism responsible for the temperature-dependent recovery of force was attributed to reduced glycogen resynthesis observed with muscle cooling, which decreased sarcoplasmic reticulum Ca^{2+} release (Cheng et al., 2017b). It is important to place into context these findings from mice muscles given that complete glycogen resynthesis can occur within 1h following fatigue induction and muscle cooling was previously applied throughout this recovery period in these mouse experiments. In humans, complete glycogen resynthesis can take 24-72h but usually CWI is only performed immediately post-exercise for 5-30 min. What remains unclear is whether 5-30 min of CWI is sufficient to blunt glycogen resynthesis and consequently affect the recovery of muscle strength and power in humans given that muscle temperature is minimally affected by short (5-10 mins) CWI immersions (Parouty et al., 2010; Jakeman et al., 2009; White et al., 2014). Another confounding factor is that muscle temperature is more difficult to manipulate in large muscle groups like the quadriceps (Broatch et al., 2014).

Thus, whether CWI enhances or is potentially deleterious to the recovery of muscle strength or fatigue resistance depends on the systemic (i.e., thermoregulatory) or local (i.e., muscle glycogen) effects of the cooling as well as the duration of CWI, with shorter CWI durations potentially functioning through a thermoregulatory action in improving muscle blood flow when exercising in hot environments whereas longer durations of CWI can blunt muscle glycogen resynthesis.

2.1.5 Effect of post-endurance exercise cooling on recovery at 24-72h following exercise

Other studies have indicated that CWI may have no impact on the recovery of neuromuscular function. At the longer 24-72h post-exercise recovery period, acute CWI is seen to have no effect on the recovery of maximal cycling peak power assessed (Anderson et al., 2018). CWI was also previously shown to not influence recovery of muscle strength (i.e., peak isokinetic torque & MVC torque) and jump performance at the 24-48 h post-exercise recovery period when compared to a control of passive recovery (Wilson et al., 2018; Wiewelhove et al., 2018). However, performance of a running shuttle test executed 24 h following an initial high-intensity exercise (8 sets x 3 mins) improved when the exercise was followed by CWI (15°C for 15 min) vs passive recovery (Brophy-Williams et al., 2011). Muscle glycogen stores are typically fully restored within 24-48 h after prolonged endurance exercise, and thus local cooling following glycogen-depleting exercise such as endurance exercise would unlikely affect performance of endurance bouts performed 1 day after the initial bout.

In summary, CWI appears to have a positive effect on the acute recovery of muscle fatigue resistance at ≤1 h post-CWI, but no effect on muscle fatigue resistance between 1-72h post-CWI (see Figure 2). With regards to neuromuscular function, CWI has a negative effect on the acute recovery of strength and power at ≤1 h post-CWI, but no effect on the recovery of

strength and power at 1-72h post-CWI (see Figure 2). While many of these previous studies performed only a short interval of 5–15 min CWI, one study with prolonged 2 h post-exercise cooling showed a negative effect on fatigue resistance as well as the recovery of muscle force (Cheng et al., 2017b). There seems to be a gap in research identifying adequate temperatures and recovery periods at which acute local cooling can be executed in order to provide potential recovery benefits. More research and experiments are clearly needed to elucidate the influence of post-endurance acute local cooling on skeletal muscle function and endurance performance.

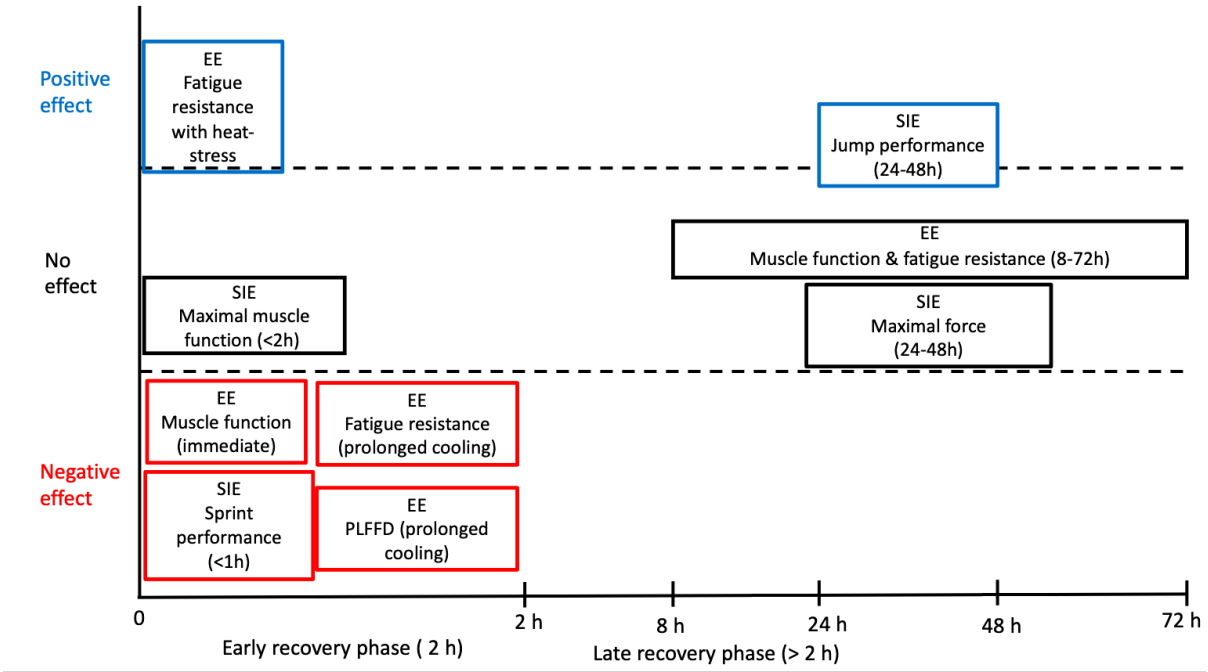

Figure 2. Summary of the main effects of post-exercise cooling on neuromuscular function, fatigue resistance and exercise performance
EE: endurance exercise; SE: sprint exercise. The blue boxes and red boxes illustrated the specific effects of cooling. Dashed blue boxes mean that the specific effect of cooling is likely.

2.1.6 Effects of local cooling on skeletal muscle recovery following sprint interval exercise (SIE):

Sprint interval exercise (SIE) is characterized by short-duration exercise that consists of 4-6 bouts of "all-out" effort lasting up to 1 min, followed by a 3-5 min rest period (maximum of

20 minutes per total session) (Wingo et al., 2018). Due to the strenuous nature of SIE, one session of SIE can result in large fatigue-induced reductions in skeletal muscle force and power generation. The specific fatigue-related mechanisms reducing muscle force generation during SIE include an increase in production of ROS which impairs excitation-contraction coupling processes, altering contraction kinetics and ultimately reducing force-generating capacity (Place et al., 2015; Powers et al., 2008; Kendall et al., 2002; Bruton et al., 2008; Lindsay et al., 2021). Additionally, SIE can increase the accumulation of metabolites such as lactate, hydrogen and inorganic phosphate ions, as well as decrease cytosolic [ATP] due to high metabolic demand of the exercise (Karatzaferi et al., 2001; Yanagisawa et al., 2003; Place et al., 2015; White et al., 2014; Westerblad et al., 2010). Although lactate is not a cause of muscle fatigue per se (Westerblad et al.,2010), specifically, the increase in concentrations of hydrogen and inorganic phosphate ions as well as the low cytosolic [ATP] can contribute to fatigue-induced decrease in contractile function (Karatzaferi et al., 2001; Cheng et al., 2017a). However, clearance of metabolites and full restoration of cytosolic [ATP] is typically achieved within 15 min of recovery, whereas a long-lasting depression of force can persist for up to 24 h after SIE, which is referred to as PLFFD (Place et al., 2015; Cheng et al., 2020). Recent studies performing high-intensity exercise have postulated a RONS-dependent mechanism responsible for PLFFD due to reductions in SR Ca^{2+} release and myofibrillar Ca^{2+} sensitivity associated with oxidative modifications to key proteins at these cellular sites (i.e., ryanodine receptor 1, troponin I) (Cheng et al., 2015). Furthermore, treatment of muscle with antioxidants and oxidizing agents can restore both SR Ca^{2+} release and myofibrillar Ca^{2+} sensitivity by shifting the intracellular redox state, supporting the hypothesis that PLFFD induced by high-intensity type exercise is RONS-dependent (Cheng et al., 2015). It is plausible that implementation of acute cooling following

SIE may augment recovery from fatigue although the exact mechanisms by which acute cooling may alter the recovery of muscle function following SIE are not known (Hyldahl et al., 2020). Given that the mechanisms underlying PLFFD in SIE are different from those of endurance or resistance exercise (Cheng et al., 2020), CWI may also act via different mechanisms to alter the recovery of neuromuscular function following SIE, which will be discussed further below.

Local acute cooling methods may be beneficial for recovery from high intensity interval exercise based on previous evidence that lowering intramuscular temperature also decreases ROS generation (Poel et al., 2008). It has been shown that exposing rat skeletal muscle to high temperatures leads to high rates of ROS generation (Edwards et al., 2007) whereby exposure of skinned rat muscle fibers to room temperature (22°C) and then to physiological temperature (37°C) reduced specific force generation due to ROS-induced force depression at higher temperatures (Poel et al., 2008). Additionally, ROS generation (i.e., by NADPH oxidase) in isolated mouse skeletal muscle fibres remains elevated throughout a 20 min recovery period following a stimulation that mimics high-intensity exercise and which does not return back to baseline (Pal et al., 2013). Due to the continued elevation of ROS generation in the immediate post-exercise recovery period in skeletal muscle, this is the premise in which CWI might reduce oxidative stress: cooling skeletal muscle in the immediate post-exercise recovery period may decrease ROS production and subsequently restore contractile force generation. Selecting SIE as a form of exercise that induces oxidative stress can be used to establish task-dependent effects of using CWI as a recovery intervention and it will also aid in our understanding of the application of CWI as a recovery modality in sports involving brief bursts of high-intensity exercise (e.g, ice hockey, basketball). Below, we will discuss the existing literature on the influence of post-exercise local cooling on the recovery of skeletal muscle

performance and function following acute SIE. For a summary of these studies, refer to Figure 2 and Table 2.

2.1.7 Effects of post-SIE cooling on immediate recovery of muscle function

Parouty et al. (2010) showed that 5 min of whole-body CWI exposure performed 5 min after a 100 m maximal swimming sprint significantly reduced subsequent 100 m sprint time executed 30 min after the first maximal swimming sprint, indicating an improvement in the recovery of fatigue resistance with CWI. With respect to muscle function however, studies have shown that implementation of CWI immediately or within 30 min following SIE has a potential negative effect on skeletal muscle function (Chow et al., 2018; Parouty et al., 2010; Crampton et al., 2014). In each study, lower-body and whole-body cooling techniques were used, the length of time for CWI were similar yet the specific muscle group exposed to CWI differed. Evidence has also indicated that lower-body CWI decreased peak and mean power after a 30 min recovery period following acute bouts of cycling sessions (3 Wingate's 3 sets x 30 s with 4 min rest between Wingates for each exercise session) (Crampton et al., 2014). All these studies showed an improvement in the recovery of fatigue resistance but reduction in strength and power immediately following CWI. It is presumed that a reduction in intramuscular temperature from CWI underlies the decrement in neuromuscular function caused by decreases in muscle perfusion, muscle oxygen extraction and oxidative capacity (Yanagisawa et al., 2012; Hydahl et al., 2020) Unfortunately, intramuscular measurements were not performed in the mentioned studies, therefore, such assumptions were made in these previous studies based on evidence of intramuscular temperature changes in other past studies (Yanagisawa et al., 2007; Barnett et al., 2004; Bailey et al., 2009).

2.1.8 Effect of post-SIE cooling on recovery at 1-2h following exercise

Only a few studies have investigated the effects of post-exercise CWI on the recovery of muscle function at 1-2 h following SIE. White et al., 2014 found that CWI (10°C for 10 min) following exercise was not effective in the restoration of maximal voluntary force generation to pre-values when assessed at 1 and 2 h following maximal sprints. Additionally, a single bout of CWI (10°C for 10 min) showed no difference in the recovery of concentric knee extension strength at 45 ° per second (0.91 rad/s) measured through a 90° range of motion (Jakeman et al., 2009) nor in the recovery of time to peak isokinetic knee extension and flexion torque between CWI/treatment group and control group (room temperature for 10 min) yet CWI impaired muscle peak force generation (Chow et al., 2018). This may be because prolonged cooling or cooling at very low temperatures reduces muscle perfusion and which also reduces motor-neuron firing rates (Oksa et al., 2002). To conclude, when compared to the control (placebo) condition, post-exercise CWI was shown to be ineffective in accelerating the restoration of force generation and muscle strength at 1-2h following SIE.

2.1.9 Effect of post-SIE cooling on recovery at 24-48h following exercise

There seems to be inconsistent findings as to whether CWI affected functional outcome measures when examining the effects of local cooling on the subsequent recovery of quadriceps muscle function taken at 24-48 h following exercise. The study by White et al. (2014) indicated that CWI treatment at 10 °C for 10 min had a greater recovery of drop jump performance (measurement of maximal voluntary power) at 24 and 48h post-exercise in comparison to the control (room temperature) condition. In contrast, within the same study by White and colleagues, they showed that squat jump values, and measurements of maximal voluntary contraction (MVC) torque remained below baseline values at 24 and 48h in the post-exercise recovery period for both the CWI and control group. Therefore, CWI improved drop jump

performance in the 24-48h post-exercise recovery period but was not more effective than the control condition in altering the recovery of squat jump height or MVC torque. In another study, Broatch et al. (2014) examined whether 15 min of CWI (10.3°C) had similar effects on the recovery of MVC strength as a thermoneutral water immersion placebo (TWP, 34.7°C) following 4 bouts of 30 second "all-out" SIE with measurements taken at similar time points (24 & 48h post-SIE). Researchers found that the recovery of MVC strength were not significantly different between the two groups at any time point during the 48h recovery period (Broatch et al., 2014). Previous studies have also shown no difference in squat jump performance following CWI (9-10 min at 9-10 °C) or control interventions at 24h and 48h after intermittent shuttle sprinting was performed at room temperature (Bailey et al., 2007, Pointon et al., 2011).

The above results show that there is divergent evidence as to whether CWI elicits any difference in the recovery of neuromuscular function (as summarized in Figure 2). A major limitation of these studies is the relatively short duration of CWI (10-15 min) and modest cooling (10-15°C) that may not have effectively induced much cooling to affect the recovery response (see Appendix: Table 1 & 2). Therefore, increasing CWI duration and lowering water temperature may be an optimal way to aid with skeletal muscle recovery post-exercise. This may be due to the fact that cooling skeletal muscle reduces oxidative stress and RONS generation, which has been proposed as the mechanism underlying PLFFD following high-intensity exercise such as sprint interval exercise (Place et al., 2015). Previous studies have only performed minimal cooling at shorter period, hence cooling for longer periods would better test this hypothesis.

2.2 Overall conclusion:

In this literature review we examined how CWI can acutely affect the recovery of skeletal muscle function following endurance and SIE. Evidence from its application to endurance exercise appears to show a beneficial effect of CWI on the recovery of fatigue resistance immediately and at 24 h following CWI treatment, whereas CWI is detrimental on the recovery of maximal strength and power immediately following endurance exercise, at 2h post-exercise, and with no effect at 24-72h post-exercise, with the results of these studies summarized in (see Figure 2).

With respect to the use of CWI following SIE (see Figure 3), some controversy exists as to whether there is any efficacy in the use of acute CWI on neuromuscular recovery at 24-48h post-exercise. Nonetheless, what may affect the broad interpretation of previous findings is that CWI has been performed over different temperature ranges and durations, and with various exercise modalities, types of CWI methods, muscle groups, and functional outcome measurements. For instance, it was previously shown that 10-15 min of CWI at 10 °C in a large muscle mass like the quadriceps will only decrease muscle temperature by 3-5 °C (Broatch et al., 2014) Furthermore, another major limitation of previous studies is that maximal effort contractions (i.e., MVC torque, drop jump, squat jump) are less sensitive indicators of fatigue than submaximal contractions which can remain impaired for >24h (i.e., PLFFD) (Place et al., 2015; Cheng et al., 2020). Thus, the temperature-dependent effect as well as the detection of fatigue may have been underestimated in previous studies.

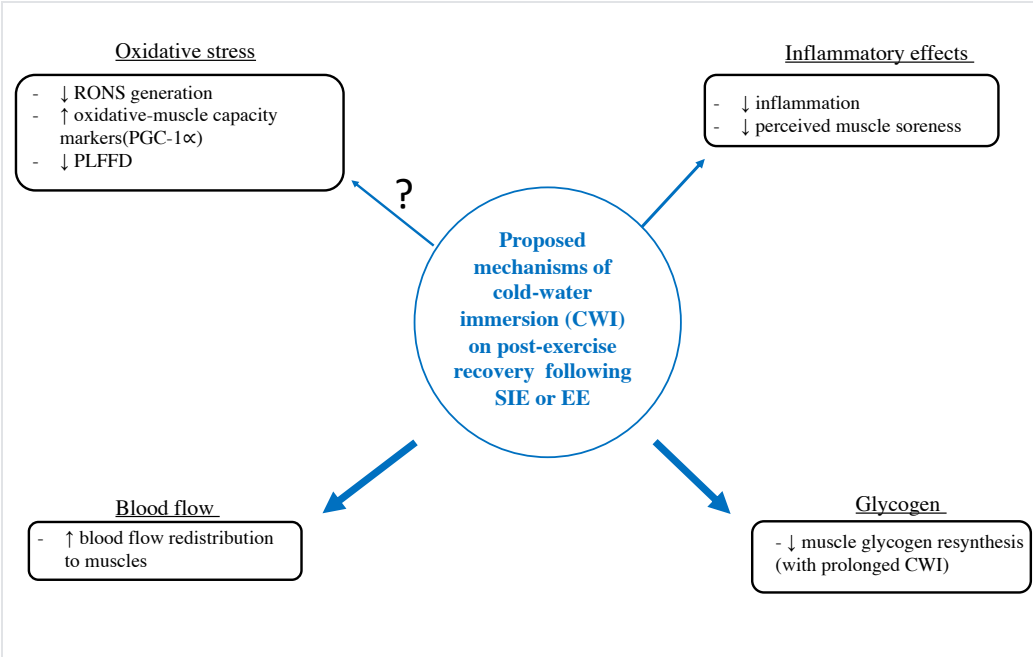

Figure 3. Proposed mechanisms of cold-water immersion (CWI) on post-exercise recovery following sprint-interval exercise (SIE) or endurance exercise (EE).
Thicker arrows represent strongly supported proposed mechanisms while thinner arrows represent weakly supported proposed mechanisms. Question mark above arrows indicate speculative evidence on CWI on post-exercise recovery following SIE or EE.

It is unclear what fatigue-related factors are contributing to neuromuscular recovery with acute CWI implementation, and clearly further studies are needed to elucidate whether CWI acts as an effective recovery modality under various conditions. Our study aims to specifically examine how effects of intense exercise (SIE), such has increased oxidation, prolonged force depression and skeletal muscle performance recover when using CWI as a recovery intervention. The novelty of my study includes 1) extreme cooling (5°C) to better test the hypothesis that cooling per se can alter post-exercise recovery processes 2) conducting the experiments on a smaller muscle group (i.e., dorsiflexors) that will be easier to cool intramuscularly compared to the quadriceps as used in previous studies, 3) this muscle will be cooled for a longer period of time (30 min instead of 10-20 min in the previously mentioned studies), and 4) further examining how CWI impacts neuromuscular function post- SIE by looking at a more sensitive indicator of fatigue such as PLFFD.

CHAPTER 3: RATIONALE AND HYPOTHESIS

Rationale:

It has been shown that intense levels of exercise increase reactive oxygen and nitrogen species (ROS) generation to the greatest extent in skeletal muscle (Sakellariou et al., 2013), and RONS have also been implicated as a potential major cause of PLFFD (Cheng et al., 2016). Reducing muscle temperature has been shown to decrease ROS generation, and consequently improve muscle force generation (Poel al., 2008). It is impractical to try and reduce muscle temperature during exercise as this will directly impact exercise performance, whereas the real-world scenario among athletes is to apply CWI immediately post-exercise. A previous study has shown that RONS generation remains elevated during the 30 min post-exercise recovery period (Pal et al., 2013), suggesting that CWI may help in reducing oxidative stress if applied for the 30 min post-exercise recovery period directly after SIE.

In the current study, PLFFD was examined in the dorsiflexors of healthy males by measuring torque from evoked stimulation at both low- (8 Hz) and high- (100 Hz) frequency before, immediately after and at 24h post-exercise. SIE involved 2-6 sets of all-out 30s isotonic dorsiflexion contractions at 30% of the maximum voluntary contraction (MVC) torque load until task failure when the electrically evoked 8:100 Hz torque ratio (i.e., PLFFD) reached 30%. A crossover design was implemented in the post-exercise recovery period with the right or left leg randomly assigned to either the treatment condition (30 minutes of CWI at 5°C immediately following SIE) or the control condition (30 min at room temperature immediately following SIE).

Objectives:

The aim of this study is to determine the effects of CWI on recovery of skeletal muscle force generation and its contributions to either central or peripheral fatigue after SIE.

Hypothesis:

It is hypothesized that CWI may be beneficial as it could mitigate the recovery of muscle force generation by reducing muscle temperature which thereby lowers oxidative stress and decreases the extent of PLFFD. Additionally, CWI is thought to reduce negative effects of central fatigue and peripheral fatigue (reductions in VA and MVC) by decreasing perceived voluntary effort and restoring muscle force output, respectively (White et al., 2014; Broatch et al., 2014).

CHAPTER 4: MATERIALS AND METHODS

4.1 Participants

A randomized cross-over study design was used in this study, in which 5 healthy, recreationally active male adults were initially recruited (19-40 years of age, BMI <30 $kg \cdot m^2$). Randomization of either CWI or CON leg was performed by alternating with every other subject for the right leg to be the CON or CWI treated leg (while the left leg would be either the CWI treated or CON leg). Individuals had normal blood pressure and did not take medication. Males with a history of any disease, injury and who trained more than 5 hours per week were excluded from the study. Caffeine consumption was avoided on the day of testing. The average height and weight were 171.7 ± 2.9 cm and 83 ± 7.9 kg, respectively. Experiments were performed at the University of Guelph (Ontario, Canada). The study protocol was approved by the Research Ethics Boards at the University of Guelph (NPES 19-06-002) and York University (e2020-001) and conformed to the standards of the Canadian Tri-Council Research Ethics guidelines. Participants were informed of the experimental procedures and gave their written consent prior to participation.

4.2 Experimental overview

Experiments consisted of three separate laboratory visits. The first visit was a familiarization trial for the participants to gain an understanding of the experimental protocol. The second visit consisted of the main experimental trial where sprint interval dorsiflexion exercise was performed in the right and left leg, with a randomized order of testing between legs. In the immediate post-exercise recovery period, the treatment leg was placed in cold water immersion (CWI) for 30 min, or the control (CON) leg kept at room temperature for 30 min post-exercise. The third visit took place at ~ 24h post-exercise for the assessment of skeletal muscle function in both the CWI and CON leg.

4.3 Experimental set-up

A HUMAC NORM isokinetic dynamometer (CSMi Medical Solutions, Stoughton, MA, USA) was used for implementing the dorsiflexion exercise protocols in humans, and to record all the torque, position, and velocity values throughout the experiment. At the start of each experimental session, skin locations were shaven and cleaned with alcohol as silver-silver, chloride (AgCl/AgCl) gel electrodes (1.5 cm x 1.0 cm; Kendall, Mansfield, MA, USA) were placed on top of the dorsiflexor and plantar flexor muscles. Two gel-coated electrodes were placed over the tibialis anterior muscle belly, the main dorsiflexor muscle, in order to induce involuntary electrical stimulation of the muscle. The participants were seated with either their right or left hip at 110° of flexion and the right or left knee at 140 ° of extension. Any movement from the torso was restricted with a 4-point seatbelt harness. The right or left foot was fixed to the dorsiflexor dynamometer foot pedal with a nonelastic tightening strap positioned over the ankle and another strap tightened at the mid-portion of the foot. The maximum ankle dorsiflexion angle was set to 130° to allow 40° joint range of motion to optimize plantar flexion stretch to ensure proper range of motion during the exercise. Silver-silver chloride (Ag/AgCl) electrodes (1.5 Å ~ 1 cm: Kendall, Mansfield, MA, USA) were used for recordings (Figure 4).

Figure 4: Experimental set-up. Image displaying the dynamometer set-up for the experimental protocol taken at the University of Guelph

4.4 Baseline voluntary and involuntary muscle function assessment

To normalize the voluntary EMG amplitudes, maximal compound muscle action potentials (M_{max}) were recorded from the tibialis anterior muscles. This was done by stimulating the deep fibular nerve via a clinical bar electrode that was covered in conductive gel. The deep fibular nerve was found by locating the head of the fibula and moving both laterally and posteriorly. Peripheral nerves were evoked by single pulse (i.e., twitch) from a constant high current voltage stimulator (model DS7AH; Digitimer, Welwyn Garden City, UK). The pulse width was set to 1s and the voltage was set to 100 V. The current was gradually increased until an M_{max} peak-to-peak amplitude plateau appeared. The M_{max} peak-to-peak amplitude measurement was also used to determine sarcolemmal membrane excitability over the course of the experiment. Thereafter, maximal voluntary strength was assessed by having participants perform 2 brief (3 s) maximum voluntary contractions (MVCs) with 5 min rest in between each MVC, with the peak value obtained from these MVC trials. To normalize the 100 Hz evoked tetani for 1s, the stimulation was gradually adjusted to ensure that the 100 Hz torque was ~30% of MVC torque. Single pulse width was set to 100 μs and voltage to a maximum of 400V. To determine voluntary activation (VA) using the interpolated twitch technique, 100 Hz tetani were superimposed on the plateau portion of the MVCs, followed by a 100 Hz tetanus evoked at rest. Participants were verbally encouraged to contract as forcefully as possible during each MVC. Percentage VA was calculated using the following equation VA% = (1 - interpolated twitch torque/ resting twitch torque) x 100 (Gandevia et al., 2001). All participants were required to reach at least 95% VA before moving toward with the rest of the experimental protocol. MVCs were followed by two isotonic contractions at 30% of MVC load to determine peak power. The highest value of the MVCs was used for further analyses in the procedures.

Before starting the fatiguing protocol, a torque-frequency curve was obtained in order to establish baseline measurements (PRE) of involuntary evoked torque. The stimulation intensity here was 30% of MVC torque as previously determined with 100 Hz tetani. Peak torque at 8 and 100 Hz were obtained, and the ratio of 8 to 100 Hz torque was calculated at baseline in order to assess PLFFD following fatigue induction.

4.5 Maximal rate of torque development and maximal rate of torque relaxation (MRTD & MRR) measurements:

PRE, POST and 24h values of torque values at 8 and 100 Hz were obtained using LabChart software to calculate maximal rate of torque development (MRTD) and maximal rate of torque relaxation (MRR). Similar to calculations of MRTD & MRR in Cheng and Rice (2012) study, MRTD & MRR were calculated by obtaining maximal time to peak slope for either the control or CWI group and dividing the value by the maximal torque value. MRTD and MRR of evoked 100 Hz tetani were then normalized by dividing the peak of change of torque $N\cdot m\cdot s^{-1}$) by the peak torque of the 100 Hz value (N·m) (Cheng and Rice, 2012).

4.6 Fatigue and recovery protocol

Exercise involved single-legged concentric isotonic contractions of the dorsiflexor muscles at 30% of MVC load. Repeated sets of 30 reps of all-out effort were performed until task failure, which was defined as the timepoint when the ratio of 8 Hz:100 Hz torque assessed in the rest interval between sets was reduced to 30% of the value at PRE. The rationale for normalizing the extent of submaximal torque reduction was to ensure that the extent of fatigue was similar at task failure between the CWI and CON leg prior to employing the different post-exercise recovery interventions. Immediately after task failure (POST), PLFFD was assessed by stimulating with a 8 and 100Hz tetanus, followed by a 3-5s MVCs with interpolated twitch technique employed, followed by two isotonic contractions at 30% of the isotonic load. Thereafter, in the post-exercise recovery period, the CON leg recovered at room temperature (i.e., no temperature intervention) while the CWI leg was fully immersed in a 5°C ice water bath

(consisting of cold water and ice) up to the tibial tuberosity for 30 min. In order to test a temperature-dependent effect of CWI, this temperature and duration of immersion were chosen as they represent a more extreme cooling condition than chosen in previous studies. The CWI leg was kept dry by placing the leg in a plastic bag prior to water immersion and all air was compressed out of the bag during immersion of the leg in the ice water bath. At the 24 h post-exercise recovery period, participants returned to the lab and the same neuromuscular measurements were taken as those at baseline (PRE) to assess any potential differences between conditions in the recovery of neuromuscular function (Figure 5).

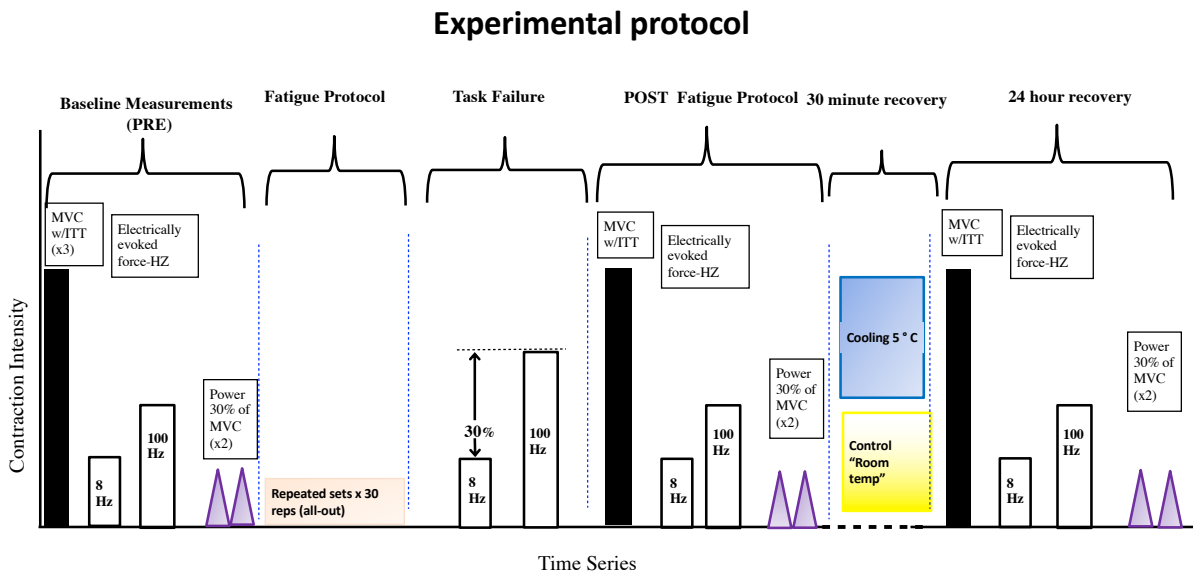

Figure 5. Experimental protocol. Schematic representing an outline of the experimental procedures during one session.

4.7 Statistical analysis

All results are expressed as mean ± SEM. The level of significance was established at $p < 0.05$ for all analyses. A two-tailed paired t-test was used to test differences in the number of contractions during the fatiguing protocol between the CWI and control leg within subjects. Two-way repeated measures ANOVA and posthoc analysis tests (both Dunnett's and Sidak's) were used to examine differences of all other measurements between the CWI and control group over time (torque-frequency relationship, voluntary activation, maximal voluntary contractions, peak power and 8:100 Hz torque ratio) (GraphPad Prism Software, USA).

CHAPTER 5: RESULTS

5.1 Baseline Measurements, Fatigue Protocol, and Voluntary Activation

5.1.1 Contraction number during fatigue protocol

Paired t-test was used to analyze the contraction numbers (repetitions) for each set during the fatiguing protocol between the CWI and control leg to reach task failure. No significant difference in contraction numbers was found between the two legs ($p=0.3739$) (Figure 6), indicating similar fatigability between the CWI and control leg.

5.1.2 Decline in total peak power output during fatigue protocol

The decline in peak power output during fatigue stimulation between both legs of each subject (CWI and control leg) is shown in Figure 7A and B. There was a significant decline in peak power output in both legs during the first and last set ($p<0.05$), but there was no difference in the amount of decline in power output, indicating similar fatigability between the CON and CWI leg.

5.1.3 Voluntary activation in CWI and control

Near-maximal voluntary activation was seen between both the CWI and control leg before the fatiguing protocol (PRE), immediately post (POST) and 24h after SIE (Figure 8). Specifically, mean values for voluntary activation of CWI and control leg throughout the experiment were at 96.9 ± 0.3% and 96.5 ± 1.3, respectively. Thus, central fatigue did not contribute to the fatigue-induced decline in torque or power following SIE. Furthermore, there was no significant difference in voluntary activation between the two groups at any timepoint ($df=1, F\ ratio=1.695, p=0.13$). This indicates that the subjects were able to fully activate their muscle voluntarily within all periods of the protocol.

5.2 Neuromuscular fatigue immediately post- SIE:

5.2.1 Maximal voluntary contraction torque

There was a decline in maximal voluntary contraction (MVC) torque in both the CWI and control leg in comparison to values at PRE ($df=2, F\ ratio=21.75, p<0.05$). MVC torque

between the CWI and control were not statistically different at POST (*df=2, F ratio=3.2, p=0.069*) (Figure 9). This indicates that the fatigue-induced decline in maximal voluntary strength is not different between the control and CWI leg immediately post-SIE.

5.2.2 Peak power

Peak power values immediately post-SIE between the CWI and the control leg were significantly reduced in comparison to peak power values at PRE *(df=2, F ratio=21.8, p < 0.05)*, but there was no difference in peak power values between the CWI vs. control leg (df=1, F ratio=0.78, *p=0.39).* This signifies that the fatigue-induced decrease in peak power is not different between the control and CWI legs immediately post-SIE (Figure 10).

5.2.3 M-wave amplitude

To determine whether sarcolemmal membrane excitability was impaired by fatigue, M-wave amplitude was assessed at task failure. M-wave amplitude immediately post-SIE between the CWI and control leg were not significantly reduced compared to the PRE value (df=2, F ratio=0.16, *p=0.83*), with no difference observed for M-wave amplitude between groups (Figure 12). Thus, there was no evidence of impaired sarcolemmal membrane excitability following SIE.

5.2.4 Recovery of submaximal force (PLFFD)

Prolonged low-frequency force depression (PLFFD) is induced by fatiguing exercise and can be observed as a greater depression in submaximal compared with maximal torque immediately following fatigue induction. In the current study, PLFFD (expressed as a ratio of 8 Hz torque relative to 100 Hz torque) was significantly reduced immediately post-SIE when compared to PRE values *(df=2, F ratio=14.03, p<0.05)* (Figure 11). The extent of PLFFD reduction between both the CWI and control leg was not significantly different *(df=1, F ratio =<0.001, p=0.47).*

5.2.5 Maximal rate of torque development (MRTD)

Maximal rate of torque development (MRTD) values immediately post-SIE between the CWI and control leg were not significantly reduced in comparison to the PRE MRTD values (*df=2, F ratio=3.04, p=0.07*), with no difference seen between both groups (Figure 13).

5.2.6 Maximal rate of torque relaxation (MRR)

Maximal rate of torque relaxation (MRR) values immediately post-SIE between the CWI and control leg were not significantly reduced in comparison to the PRE MRR values (df=2, F ratio=0.66, *p=0.53*), with no difference seen between both groups (Figure 14).

5.3 Neuromuscular recovery at 24h post- SIE

5.3.1 Maximal voluntary contraction torque

Compared with the PRE value, MVC torque values between both the CWI and control leg recovered completely at 24h post-exercise (*p=0.36* for CWI leg *vs. p=0.44* for control leg*)*, with no difference between the groups (Figure 9).

5.3.2 Peak power

Peak power at 24h post-SIE recovered back to the PRE value, with no significant difference observed for the peak power between groups (*p=0.87* for control vs. *p=0.22* for CWI leg) (Figure 10).

5.3.3 M-wave amplitude

M-wave amplitude 24h post-SIE was not significantly different from the PRE value, with no significant difference observed for M-wave amplitude between groups (*p=0.87* for control vs. *p=0.91* for CWI leg) (Figure 12).

5.3.4 Recovery of submaximal force (PLFFD)

PLFFD (expressed as a ratio of 8 Hz torque relative to 100 Hz torque) was significantly reduced at 24h post-exercise compared with values at POST and at PRE *(p<0.05).* This indicates that submaximal force generation (i.e., PLFFD) remains depressed long after the SIE session (Figure 11). However, there was no difference in the extent of PLFFD between the control and CWI condition at 24h post-SIE.

5.3.5 Maximal rate of torque development (MRTD)

There was a significant depression in the MRTD values in only the CWI leg in comparison to control leg values at 24h post-SIE (*p=0.47* for control vs. *p<0.05* for CWI*)*. However, there was no difference in the rate of torque development between the CWI and control groups (Figure 13). This indicates that acute CWI affects the recovery of maximal rate of torque development for a prolonged period.

5.3.6. Maximal rate of torque relaxation (MRR)

There was no significant difference between the maximal rate of relaxation values at 24h post-SIE compared with PRE (Figure 14), nor was there a significant difference between the CWI and control leg MRR at 24h post-exercise (*p=0.54* for control vs. *p=0.98* for CWI leg) (Figure 9).

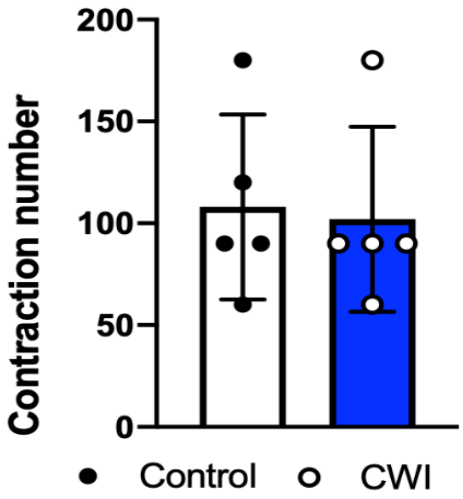

Figure 6: Contraction number during fatiguing protocol. The number of contractions for each set comparing the control and CWI leg during the fatiguing protocol, p=0.3739. White bars and black circles represent the control leg, blue bars white circles represent CWI leg. Data are shown as mean ±SEM with n=5/group.

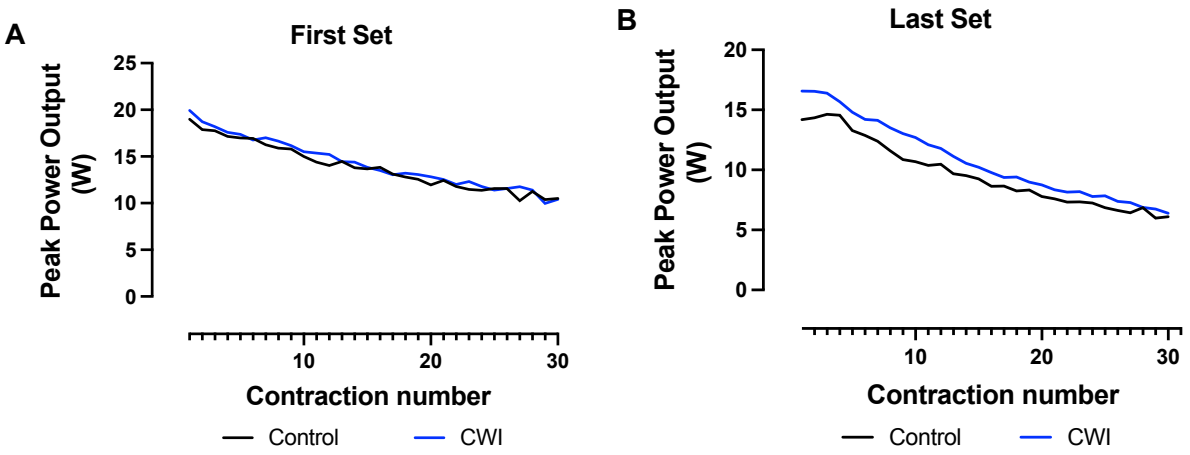

Figure 7. Fatigue-induced decline of peak power output in first and last set of SIE. A) Peak power output represented in absolute values for the 30 contractions of the 1st set between control (black line) and CWI leg (blue line). **B)** Peak power output represented in absolute values for 30 contractions of last set between control and CWI leg. Data are shown as means ± SEM, n=5/group.

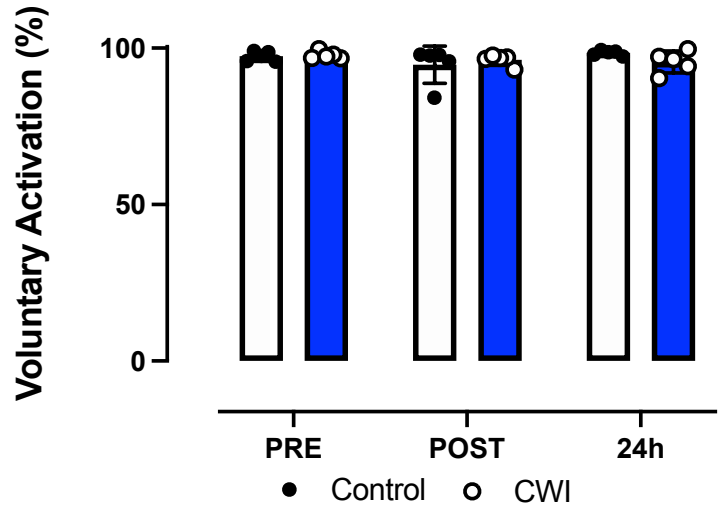

Figure 8. Voluntary activation before and after SIE. Near maximal voluntary activation shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group.

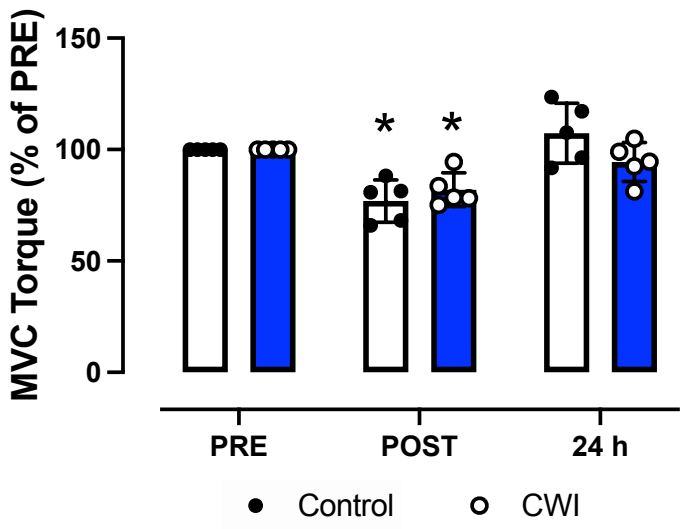

Figure 9. Maximal voluntary contraction torque before and after SIE. Maximal voluntary activation shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group. * indicates significantly different from the value at PRE.

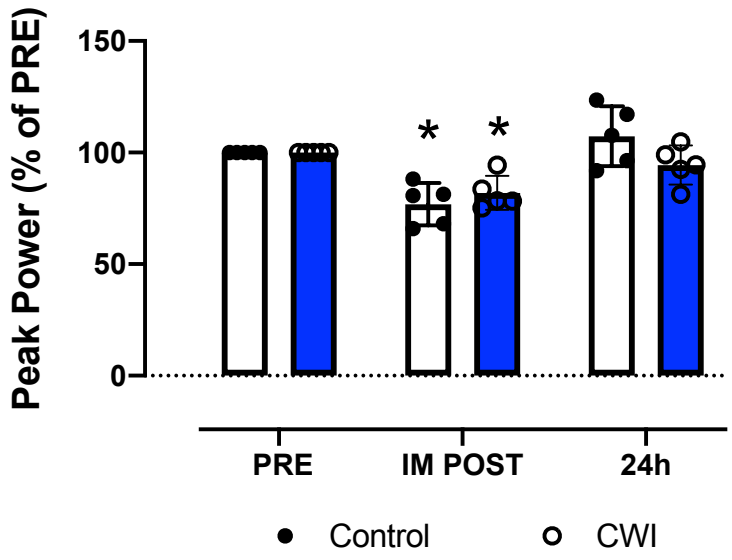

Figure 10. Peak power before and after SIE. Peak power shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group. * Indicates significantly different from the value at PRE.

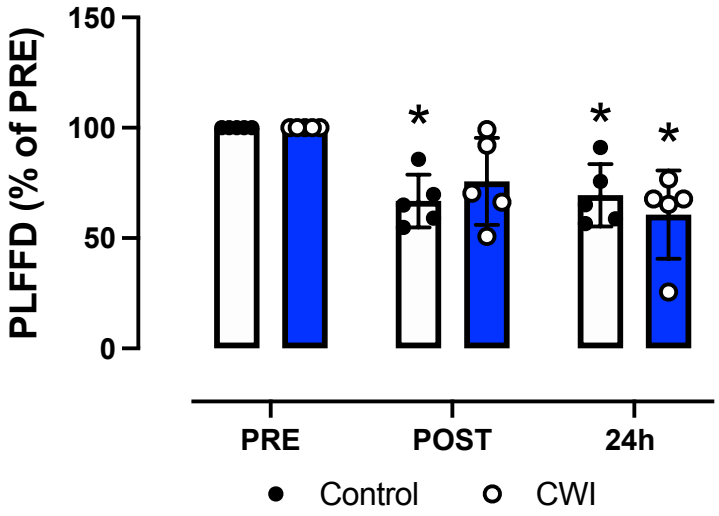

Figure 11. Prolonged low-frequency force depression (PLFFD) before and after SIE.
PLFFD shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group. * indicates significantly different from the value at PRE.

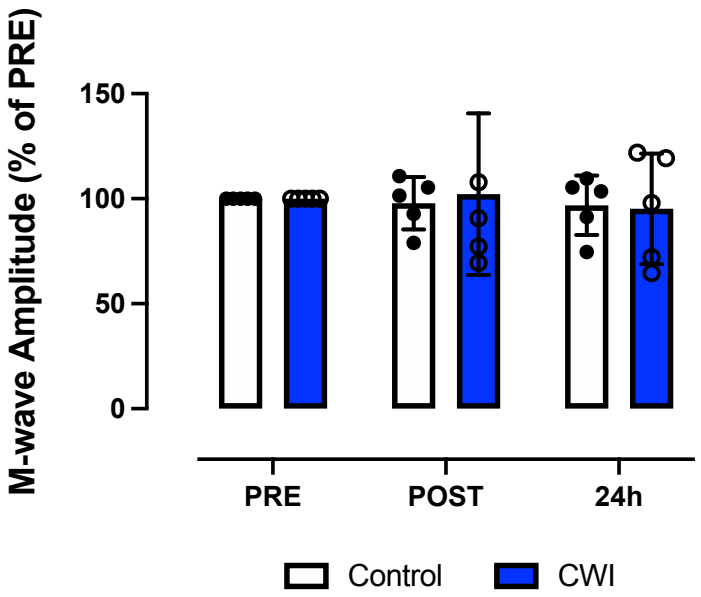

Figure 12. M-wave amplitude before and after SIE.
M-wave amplitude shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group.

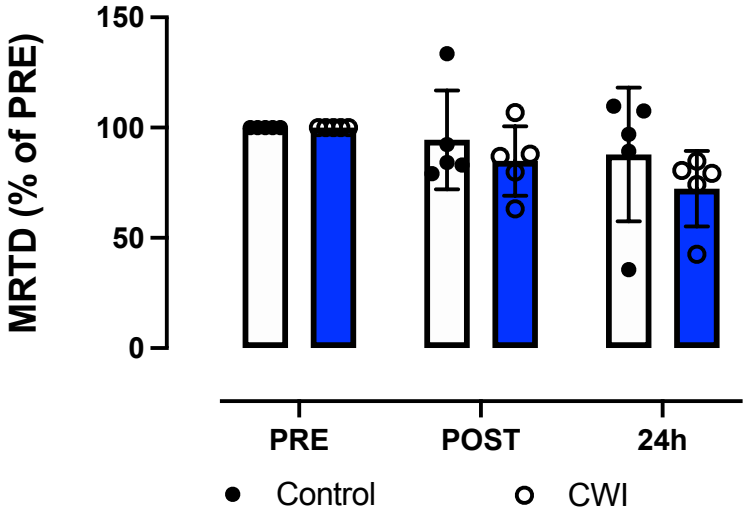

Figure 13. <u>Maximal rate of torque development (MRTD) before and after SIE.</u>
MRTD shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group.

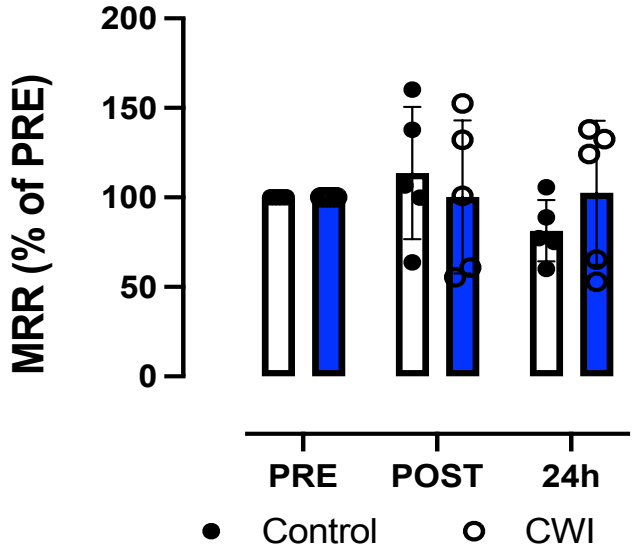

Figure 14. <u>Maximal rate of torque relaxation (MRR) before and after SIE.</u>
MRR shown at PRE, POST and 24h post-SIE between the control (white bars, black circles) vs CWI leg (blue bars, white circles). Data are shown as means ± SEM, n =5/group.

CHAPTER 6: DISCUSSION

The primary purpose of this study was to examine whether CWI influences the recovery of skeletal muscle force generation and how it effects either central or peripheral fatigue after sprint-interval exercise (SIE). The results from this study showed that the fatigue-induced reduction in MVC and peak power by SIE was primarily attributed to intramuscular factors. Specifically, there was no evidence of central fatigue or impaired sarcolemmal membrane excitability, but there was evidence of muscle fatigue shown as impairments in electrically evoked force (i.e., PLFFD). Furthermore, these results show that even though SIE induced long-lasting PLFFD that persisted through to the 24h post-exercise recovery period, CWI did not alter the recovery of PLFFD. In conclusion, 30 min of CWI in a 5°C ice water bath was not effective at accelerating the recovery of neuromuscular function at the 24h post-exercise recovery period following SIE.

The paired experimental design of this study involved SIE performed on the right and left dorsiflexors of subjects, with a randomization as to which leg received the CWI treatment and which was the control leg. Although differences in fatigability between the legs due to leg dominance could have confounded the results in the current experiment, there were no differences across any neuromuscular function parameters (i.e., voluntary activation, M-wave amplitude, MVC torque, peak power, PLFFD, MRR, MRTD) when comparing the PRE to POST-SIE values. Thus, this aspect of the study design had no influence on the outcome of the current experimental results.

With regards to the CWI recovery intervention, all past studies have typically employed CWI immediately at the end of an exercise task, which matches the real-world scenario as to when

CWI is typically used as a recovery intervention in sports events (see Table 1, 2). In this study, the CWI protocol was executed immediately post-SIE with the hypothesis that cooling reduces the impact of oxidative stress and decreases the extent of PLFFD. For the CWI intervention, we also employed a longer duration and greater cooling condition than previously used in past studies to better assess the potential effects of cooling, if any, on the post-exercise recovery of neuromuscular function. Although intramuscular temperature was not measured, pilot testing showed that the CWI intervention was sufficiently cold to numb many sensations in the leg, and which substantially impaired sarcolemma membrane excitability, electrically stimulated torque, and maximal voluntary torque at the 1h post-exercise recovery period. Nonetheless, one of the main interests of this study was to assess neuromuscular function at the 24h post-exercise recovery period, which translates to the real-world situation where athletes are employing CWI in attempt to recover faster between multi-day training sessions or multi-day competitions. My results show that there was no difference in the recovery of MVC, peak power and PLFFD between the CWI and control group (Figure 9, 10, 11). Previous studies that performed CWI post-SIE have also shown similar results undergoing less strenuous CWI treatment at ~10 °C for 15 min, whereby the recovery of MVC strength at 24 h post-SIE was not different compared with the thermoneutral (34.7°C) water immersion placebo condition (Broatch et al., 2014). White and colleagues (2014) also demonstrated that CWI for 10 or 30 mins at 10 °C showed similar recovery of squat jump performance when compared to CON at 24 and 48 h post-SIE (White et al., 2014). On the other hand, within this same study, CWI for 10 or 30 min at 10°C showed greater recovery of drop jump height when compared to CON or more modest CWI at 20°C temperature for 10 or 30 min (White et al., 2014). White and colleagues (2014) suggested that CWI may be helping to preserve stretch-reflex sensitivity to facilitate improved drop jump

performance, although this hypothesis was not tested previously. In the current study, I speculate that CWI may not have had a significant effect on the recovery of neuromuscular function since the majority of RONS generation likely occurs during the fatiguing exercise, and this cannot be prevented by a post-exercise CWI intervention. Specifically, any effects of RONS on muscle function would already be induced by the exercise itself with minimal potential effects induced by mitigating oxidative stress in the post-exercise recovery period. Furthermore, cooling for brief periods may minimally affect the recovery of neuromuscular function over a 24 h period as muscle temperature recovers back to its thermoneutral state within ~30 min (Hyldahl et al., 2020; Yamane et al., 2006; Watanabe et al., 2018), indicating that only ~4% of the 24h recovery period involves cooling (i.e. 30 min CWI followed by 30 min recovery back to its thermoneutral state). Therefore, these studies support evidence that acute cooling following SIE has no advantageous or detrimental influence on the recovery of neuromuscular function at 24 h post-exercise.

6.1 Central contributions to fatigue and post-exercise recovery

During voluntary exercise, fatigue can be induced by impairments in the neuromuscular system at the central and peripheral level. Our results show that central fatigue did not contribute to a decline in torque or power immediately following or 24h post-SIE between both the CWI and control group. Mean values for post-fatigue voluntary activation values of both the CWI and control group were near-maximal at ~97.7 vs ~97.4%, respectively (see Figure 8). These results from the dorsiflexor muscles are consistent with those of Place et al. (2015) from the knee extensor muscles who showed using the interpolated twitch technique that central fatigue is not observed immediately following SIE. However, a study by Fernandez-del-Olmo and colleagues observed central fatigue immediately post-SIE (Fernandez-del-Olmo et al., 2013). In this study, MVC force following isometric knee extension reduced significantly between two

sets of 30s supramaximal cycling Wingates. Additionally, researchers found that after each Wingate test, voluntary activation declined from 91% to 60% (34% reduction), indicating an inability to maximally activate the motor units. This discrepancy of central fatigue contribution may be due to the fact that central fatigue measurements were taken via different techniques. For instance, Place et al. (2015) used peripheral nerve stimulation to assess voluntary activation whereas Fernandez et al. (2013) used motor cortex stimulation (TMS) (Place et al., 2015; Fernandez- del-Olma et al., 2013). TMS is more advantageous in assessing voluntary activation as it can isolate central fatigues' origin to the motor cortex. However, central fatigue can be highly overestimated when employing TMS due to inaccuracies with predicting the resting twitch force used to calculate voluntary activation with the interpolated twitch technique. With TMS, linear extrapolation is employed to predict the resting twitch force, but this extrapolation is in itself confounded by fatigue and exhibits poor linearity (Cadigan et al., 2017). On the other hand, with peripheral nerve and transcutaneous stimulation, the latter stimulation method being used in the current study, these are highly sensitive and can reveal that most healthy adults fail to fully activate skeletal muscle fibers despite exhibiting maximal effort during intense exercise (Shield et al., 2004). Furthermore, peripheral nerve and transcutaneous stimulation directly assesses the resting twitch force, leading to an accurate calculation of voluntary activation when using the interpolated twitch technique (Cadigan et al., 2017). Using transcutaneous stimulation in my study was beneficial as it provides an accurate quantification of voluntary activation, in which we showed that central fatigue is not contributing to the fatigue-induced depression in torque and power following SIE.

Additionally, M-wave amplitude data shows that there was no evidence of impaired sarcolemma membrane excitability following SIE as the M-wave amplitude at POST and 24h

following SIE displayed no significant reduction when compared to PRE values (see Figure 12). These results are consistent with those of Place et al. (2015), who showed from the *vastus lateralis* no significant change in M-wave amplitude either immediately post, 5 mins or 24h following SIE when compared to before exercise (Pre) values (Place et al., 2015). Similarly, Fernandez- del- Olmo et al (2013) showed that M-wave amplitude did not change after Wingate exercise, indicating that membrane excitability was not affected during the intense bouts of cycling and that the decrease in resting twitch amplitude was due to changes in the vastus lateralis but not in electrical transmission of motor nerve ends (Fernandez- del-Olma et al., 2013). Altogether, these findings show that fatigue following SIE cannot be explained by impairments at the sarcolemma level.

6.2 Peripheral contributions to fatigue and post-exercise recovery

Forms of high-intensity exercise, such as SIE, is associated with increased oxidative stress and impaired contractile force generation of skeletal muscle fibres following fatigue (Cheng et al., 2016; Cheng et al., 2017a; Place et al., 2015). Especially, intense anaerobic exercise has been shown to induce prolonged submaximal force depression, which ultimately reduced the perceived voluntary effort required during exercise execution (Cheng et al., 2020; Place et al., 2015). In the current study, PLFFD was observed immediately post-SIE and up to the 24h post-exercise recovery period. Such factors may be due to defects in sarcoplasmic reticulum Ca^{2+} release, contributing to peripheral fatigue (Place et al., 2015; Olsson et al., 2020). Furthermore, an increase in the production of RONS has been shown to induce long-lasting effects on sarcoplasmic reticulum Ca^{2+} release and skeletal muscle fiber contractile function (Bruton et al., 2008; Nielsen et al., 2014; Cheng et al., 2015; Cheng et al., 2016; Olsson et al., 2020). However, our data showed that the extent of PLFFD reduction between both the CWI and

control leg was not significantly different when cooling was executed immediately and 24h post-SIE (see Figure 11). As previously mentioned, it remains uncertain if post-exercise CWI is sufficient to reverse any increase in oxidative stress induced by the exercise itself. In comparison to the null effects of cooling on the post-exercise recovery of PLFFD, thermal pre-treatment (high temperature of 42 ºC) was previously shown to improve recovery in fast-twitch Type II muscle fibers in rats (Watanabe et al., 2018). Watanabe et al., demonstrated that exposing the hindlimbs of rats to heated water at 42 ºC for 20 mins before a fatiguing stimulation over a span of 5 days improved the recovery of submaximal force post-stimulation (Watanabe et al., 2018). Such results can be due to the fact that exposing fast-twitch muscle fibers to high temperatures increases ROS production (Cheng et al., 2016) which was previously suggested to alter proteins involved in the regulation of SR Ca^{2+} release by oxidizing them (i.e., RyR1). Therefore, by extension, cooling may result in opposite effects by extensively limiting RONS generation and impairing the excitation-contraction kinetics (White et al., 2014). Hence, a specific amount of RONS production and oxidative stress may be beneficial with helping to delay onset muscle fatigue and improve the adaptive responses during exercise (Radak et al., 2017; Ristow et al., 2014)

Maximal rate of torque development and rate of relaxation (MRTD & MRR) are important muscle contractile kinetic measurements that potentially explain the intramuscular contributions to the fatigue and recovery of power generation (Cheng et al., 2012). Research has shown that isotonic power is greatly depressed following a maximal-effort fatiguing task of repeated loaded shortening dorsiflexions (Cheng et al., 2012), and that decreased MRTD and increased MRR were indicative of an intramuscular contractile slowing contributing to the fatigue-induced decreased in muscle power generation. However, my results indicated no

significant reduction of both the MRTD and MRR values at POST or at 24h post-SIE between the CWI and control group. One possible explanation for these findings is that the muscle was undergoing slight recovery during the neuromuscular assessment at POST since many parameters were sequentially assessed at this timepoint (see Figures 13, 14) meaning a possible underestimation of the extent of fatigue-induced contractile slowing following SIE.

In conclusion, we show that central fatigue is not the contributing factor to decline in torque or power post-SIE. Additionally, acute cooling post-SIE has no advantageous or disadvantageous effects on the fatigue and recovery of neuromuscular function up to 24h post exercise.

6.3 Limitations

All of these experiments on the sample size of five subjects were performed before the start of the pandemic in March 2020. Thus, a major limitation affecting the outcome of our findings have been the small sample size that makes it difficult to extrapolate our findings to the general population. Another limitation is that intramuscular RONS generation was not measured in the current study, and thus I cannot confirm whether SIE does result in extensive RONS production and whether RONS generation can be blunted by CWI. Furthermore, we cannot confirm whether RONS or additional mechanisms could be responsible for the induction of PLFFD following our exercise protocol. Our MRTD and MRR measurements also appear to be compromised due to the inclusion of too many neuromuscular parameters being measured following fatigue, and which may have allowed the muscle contractile kinetics to recover, leading to an underestimated contribution of contractile slowing to the fatigue-induced reduction in power. Furthermore, another experiment that was planned but not completed was to measure intramuscular temperature while performing CWI post-SIE to determine the extent to which

CWI affected intramuscular temperature. In addition, it was noted in the literature review that a main benefit of CWI is on improving muscle fatigue resistance when a subsequent exercise bout is performed within an hour after the first exercise bout. If time allowed, it would have been interesting to test whether my severe cooling protocol would also affect muscle fatigue resistance in the acute post-exercise recovery period following SIE.

6.4 Future directions

Future directions can aim to look at passive heating as a possible recovery intervention instead of passive cooling, which may show promise as a more effective recovery intervention. Recently, pre-treatment of muscle at high temperature (~40 ºC) was shown to accelerate the recovery of PLFFD (Watanabe et al., 2018). ROS, such as superoxide, is produced during strenuous exercise which can oxidize proteins involved in excitation-contraction coupling processes. This alteration of protein structure and function due to oxidation leads to impairments in SR Ca^{2+} release and myofibrillar-Ca^{2+} sensitivity, which appear responsible for PLFFD (Bruton et al., 2008; Cheng et al., 2015). The mechanism as to why thermal pre-treatment was shown to accelerate the restoration of SR Ca^{2+} release and PLFFD is not known, but it was speculated by Watanabe and colleagues that heating the muscle may have enhanced the endogenous antioxidant enzymes in reversing the oxidized proteins (RyR1) involved in SR Ca^{2+} release (Watanabe et al., 2018). Furthermore, long-term improvements in skeletal muscle function were shown following 4-6 wks of sprint interval training (SIT) via enhanced markers of mitochondrial oxidative capacity attributed to improved mitochondrial biogenesis and mitochondrial respiratory capacity via increased content of PGC-1 α, Complex I,V and heat-shock proteins (Hafen et al., 2018). It has been shown that overexpression of a heat-shock protein (HSP60), which is activated by heat stress, also induces PGC-1α expression (Barone et

al., 2016). Therefore, it is plausible that chronic muscle heating can have potential positive effects on post-SIT skeletal muscle adaptations in comparison to CWI. Unfortunately, there is a lack of knowledge regarding the effects of heating post-SIE on skeletal muscle function/adaptations and additional studies are needed to elucidate the efficacy of heating pre-SIE on fatigued skeletal muscle.

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APPENDIX A:

Table 1: A summary of the effects of cooling following endurance exercise on neuromuscular function and performance

CMJ: countermovement jump; CON: control; CWI: cold water immersion; CWT: contrast water therapy; DJ: drop jump; ECC: eccentric; Ex: exercise; F: female; HIIE: high intensity interval exercise; M: male; KE: knee extension; KF: knee flexion; MVIC: maximal voluntary isometric contraction; PO: power output; PR: passive recovery; SMVIC: maximal voluntary isometric contraction with superimposed electrical stimulation; RE: resistance exercise; RH: relative humidity; TT: time trial; TWI: thermoneutral water immersion; Vmax: speed sustained at the end of the maximal incremental test (VO_{2max} test); VO_2: oxygen consumption; VT: ventilatory threshold; WBC: whole-body cryotherapy; wk: week; ↑: positive effect; Y: year; ↓: negative effect; Ø: no effect.

Note: water immersion was applied up to the waist/lower part of the trunk, unless stated otherwise.

Participants	Exercise protocol	Post-exercise recovery method	Main finding	Main effect	Reference
Single post-exercise exposure					
Well trained team-sport players (8 M, 21 Y).	Running: - 8x3min at 90% velocity obtained at $VO_{2max.}$.	Crossover design: - CWI: 15°C for 15 min immediately or 3 h post-Ex. - CON: 15 min PR immediately after Ex.	- Higher number of shuttles completed (Yo-Yo intermittent recovery test) **@ 24 h** post-Ex in CWI (immediately > 3 h post-Ex) vs. CON.	↑ of CWI (immediately post-HIIE) on shuttle test performance **@ 24 h**.	Brophy-Williams et al.
Recreationally trained subjects (4 F and 1 M, 26 Y).	Arm cycling: - 3x5 min (all-out) + 4x15 min arm cycling at 50% $VO_{2max.}$	Crossover design. Use of water-perfused arm cuff: - Ice-chilled for 2 h. - Heated at 38°C for 2 h. - CON: heated at ~33°C for 2 h.	- Better maintenance of mean PO (3x 5 min all-out arm cycling) **immediately** after recovery method after heating vs. cooling.	↑ of heating and ↓ of cooling on fatigue resistance **@ 0 h**.	Cheng et al.
Recreationally trained triathletes (9 M, 30 Y).	Cycling: - 5min at 50% VO_{2max} + 5min at 60% VO_{2max} + 80% VO_{2max} until exhaustion. -	Crossover design: - CWI: 15°C for 30 min. - TWI: 34°C for 30 min. - CWT: 2,5 min at 8°C and 2,5min at 40°C for 30 min. - CON: 30 min active recovery.	- Greater time to failure during intense cycling (80% VO_{2max}) **@ 5 min** post-recovery in CWI vs. other conditions.	↑ of CWI on fatigue resistance **@ 5 min.**	Crampton et al.
Recreationally trained subjects (9 M, 24 Y).	Running: - Unilateral ECC KF EX + 90 min running (70% VO_{2peak})	Crossover design: - CWI: 15°C for 15 min. - HWI: 38°C for 15 min. - TWI: 28°C for 15 min. - CON: 15 min PR.	- Similar 5-km running time **@ 4 h** post-recovery in all conditions.	Ø of CWI and HWI on endurance performance **@ 4 h.**	De Paula et al.
Well trained subjects (9 M, 29 Y).	Running: - 5min at 50% Vmax + 5min at 60% Vmax + 90% Vmax until exhaustion.	Crossover design: - CWI: 15°C or 8°C for 15 min. - CON: 15 min PR.	- Higher time to failure at 90% Vmax **@ 5 min** post-recovery in CWI (8°C only) vs. CON.	↑ of CWI (8°C only) on fatigue resistance **@ 5min**.	Dunne et al.
Recreationally trained subjects (15 M, 21 Y).	Cycling: - 12 min at 85% VT + 30s/30s interval bouts (90% peak PO/40% Peak PO) to exhaustion.	Crossover design: - CWI: 8°C for 5 or 10 min. - CON: PR.	- Higher time to failure (30s/30s interval bouts at 90% peak PO/40% peak PO) **immediately** post-recovery in CWI (5 and 10 min) vs. CON.	↑ of CWI on fatigue resistance **@ 0 h**.	McCarthy et al.

Well trained cyclists (10 M, 27 Y).	Cycling: - 90 min at 80% VT2 + 16.1 km TT.	Crossover design: - CWI: 14.3°C for 20 min. - CON: 20 min PR	- Lower MVIC and SMVIC KE torques **@ 45 and 90 min** post-Ex in CWI vs. CON.	↓ of CWI on maximal strength **@ 45-90 min**.	Peiffer et al.
Well trained cyclists (12 M, 29 Y).	Cycling: - Time-to-exhaustion test at VT1. -	Crossover design. Immersion starting 25 min post-Ex: - CWI: 14.3°C for 5, 10 or 20 min. - CON: 20 min PR	- Similar MVIC KE torque and isokinetic KE torque (240°/s) **@ 55 min** post-Ex in CWI and CON.	Ø of CWI on maximal strength **@ 55 min**.	Peiffer et al.
Well trained cyclists (10 M, 35 Y).	Cycling: - 25 min at 65% VO_{2max} + 4-km TT.	Crossover design: Immersion starting 25 min post-Ex: - CWI: 14°C for 5 min - CON: PR	- Greater 4-km TT performance (performed after 25min at 65% VO_{2max}) **immediately** post-recovery in CWI vs. CON.	↑ of CWI on endurance performance in heat **@ 0 h**.	Peiffer et al.
Well trained triathletes (7 M, 29 Y).	Running: - 7x5 min at 105% anaerobic threshold.	Crossover design. Immersion starting 10 min post-Ex: - CWI: 5x1 min at 10°C, with 1 min rest. - TWI: 5x1 min at 34°C, 1min rest.	- Similar mean PO (5-min maximal cycling effort + 6x5 min freely paced cycling) **@ 8.5 h** post-Ex in CWI and TWI.	Ø of CWI on endurance performance **@ 8.5 h**.	Rowsell et al.
Well trained cyclists (18 M, 27 Y).	Cycling: - 60 min including 8x4 min at 80% peak PO	Crossover design. Immersion starting 20 min after Ex, up to the neck: - CWI: 14°C for 5 min. - CWT: 1 min at 14°C and 2 min at 35°C for 10min. - CON: 10 min PR.	- Similar TT performance (~14 min cycling) **@ 2.75 h** post-recovery in all conditions.	Ø of CWI and CWT on endurance performance **@ 2.75 h**.	Stanley et al.
Well trained cyclists (10 M, 32 Y).	Cycling: - 15min at 75% peak PO + 15 min TT .	Crossover design: Immersion up to the neck: - CWI intermittent: 5x1 min at 10°C, 15°C or 20°C, with 2 min rest. - CWI: 20°C for 15min. - CON: active recovery	- Reduced cycling performance (15min at 75% peak PO + 15 min TT in heat) **@ 40 min** post-recovery in CON but not in CWI conditions. No differences between the CWI conditions.	↑ of CWI on endurance performance in hot environment **@ 40 min**.	Vaile et al.
Well trained cyclists (10 M, 34 Y).	Cycling: - 15min at 75% peak PO + 15 min TT	Crossover design: Immersion up to the neck: - CWI intermittent: 5x1 min at 10°C, 15°C or 20°C, with 2 min rest. - CWI: 20°C for 15min. - CON: active recovery	- Reduced cycling performance (15min at 75% peak PO + 15 min TT in heat) **@ 40 min** post-recovery in CON but not in CWI conditions. No differences between the CWI conditions.	↑ of CWI on endurance performance in hot environment **@ 40 min**.	Vaile et al.
Recreationally trained runners (31 M, 40 Y).	Running: - Competitive marathon (42,2 km).	2 groups: - CWI: 8°C for 10min. - PLA: PR with ingestion of fruit flavored drink (placebo). *exclusion of one group.	- Similar recovery of peak isokinetic KE torque (60°/s), MVIC KE force and DJ performance (reactive strength index) **@ 24-48h** post-marathon in CWI and PLA.	Ø of CWI on maximal force and jump performance **@ 24-48 h**.	Wilson et al.
Recreational runners (46 M, 30 Y).	Running: - Competitive half-marathon (21,1 km)	2 groups: - CWI: 15°C for 15min. - CON: 15 min PR. *exclusion of two groups.	- Moderate harmful effect of CWI vs. CON on CMJ performance **immediately** post-recovery, but no effects **@ 24 h**.	Potential ↓ of CWI on jump performance **@ 0 h** but not **@ 24 h**.	Wiewelhove et al.
Repeated post-exercise exposures					
Recreationally trained subjects (17 M, 23 Y).	4 wk-endurance cycling training (12 HIIE sessions): - 8-13 x 60 s at 90-110% peak PO with 75s rest.	Immersion after each training session, 2 groups: - CWI: 10°C for 15 min. - CON: PR.	- Similar improvement of 15km- cycling TT performance in CWI and CON groups.	Ø of CWI on the improvement of endurance performance.	Aguiar et al.

Well trained subjects (21 M, 20 Y).	39 day- endurance cycling training (1-2 sessions/day): - Low-moderate intensity road rides + HIIE sessions.	Immersion 4x /wk, 2 groups: - CWI: 15°C for 15 min (up to the neck). - CON: PR	- "Unclear" greater increase in 2x4-min maximal cycling effort in CWI vs. CON groups. - "Likely" higher fatigue resistance (mean power of 2nd vs. 1st 4-min maximal effort) in CWI vs. CON groups. - "Likely" greater increase in mean sprint PO in CWI vs. CON groups. - No between-group difference in 10 min TT performance.	Unclear or likely ↑ of CWI on endurance/sprint performance.	Halson et al.
Recreationally trained subjects (6 M, 20 Y).	4-wk cycling training (3-4 sessions/wk) - 5 min at 35% VO_{2max} + 25 min at 70% VO_{2max}.	Unilateral immersion after each session: - CWI: 2x 20 min at 5°C (thigh and lower leg) with 30 min rest. - CON: PR.	- Improvement of endurance performance (time of the one-leg incremental test) and VO_{2max} only in the CON leg.	↓ of CWI on endurance-training adaptation.	Yamane et al.

Table 2: A summary of the effects of cooling following sprint exercise on neuromuscular function and performance.

CMJ: countermovement jump; CON: control; CWI: cold water immersion; DJ: drop jump; Ex: exercise; F: female; KE: knee extension; KF: knee flexion; M: male; MVIC: maximal voluntary isometric contraction; PO: power output; PR: passive recovery; RH: relative humidity; SJ: squat jump; TT: time trial; TWI: thermoneutral water immersion; TWP: thermoneutral water immersion with placebo; VO_2: oxygen consumption; wk: week; Y: year; ↑: positive effect; ↓: negative effect; Ø: no effect.

Note: water immersion was applied up to the waist/lower part of the trunk, unless stated otherwise

Participants	Exercise protocol	Post-exercise recovery method	Main finding	Main effect	Reference
Single post-exercise exposure					
Recreationally trained subjects (30 M, 24 Y).	Cycling: - 4x 30s all out with 4 min rest	3 groups: - CWI: 10.3°C for 15 min. - TWI: 34.7°C for 15 min - TWP: 34.7°C for 15 min with skin cleanser added to the water (placebo).	- Greater MVIC KE torque **immediately** post-recovery, and **@ 1 h and 48 h** post-Ex in TWP vs. TWI groups, with no differences between CWI and TWP groups **immediately** post-recovery, and **@ 1, 24 and 48 h**.	Ø of CWI (vs. TWP) on maximal strength **@ 0-1 h and 24-48 h**.	Broatch et al.
Recreationally trained cyclists (10 M, 29 Y)	Cycling: - 2 x 1-km TT with 20 min rest.	Crossover design. Immersion after the 1st 1-km TT: - CWI: 14°C for 5 min. - CON: 5 min PR.	-Similar cycling time and mean cycling PO during the 2nd 1-km TT in CWI and CON.	Ø of CWI on subsequent (**@ 20 min**) cycling sprint performance.	Buchheit et al.
Recreationally trained subjects (8 M, 25 Y).	Cycling: - 3x30s all-out cycling bouts with 4 min rest (Ex1). - 35 min recovery - 3x30s all-out cycling bouts with 4 min rest (Ex2). -	Crossover design. Immersion after Ex1: - CWT1/1: 2.5 min at 8°C and 2.5 min at 40°C for 30 min. - CWT1/4: 1 min at 8°C and 4 min at 40°C for 30 min. - CON: 30 min PR	- Greater peak PO of Ex2 in CWT1/4 vs. PR, with no differences between CWT1/1 and PR. - Reduced total work during Ex2 in PR but not CWT conditions.	↑ of CWT on subsequent (**@ 35 min**) cycling sprint performance.	Crampton et al.

Well trained cyclists (8 M, 25 Y).	Cycling: - 3x30s all-out cycling bouts with 4 min rest (Ex1). - 40 min recovery - 3x30s all-out cycling bouts with 4 min rest (Ex2).	Crossover design. Immersion after Ex1: - CWI: 15°C for 30 min. - Active recovery (arm Ex) for 30 min. - CWI (15°C) with active recovery (arm Ex) for 30 min. - TWI (34°C) with active recovery (arm Ex) for 30 min.	- Lower mean PO of Ex2 in CWI vs. the other trials. Mean PO of Ex2 only preserved in arm Ex and TWI with arm Ex. - Lower peak PO of the 1st sprint of Ex2 in CWI and CWI with active recovery vs. the two other trials. Peak PO of Ex2 only preserved in TWI with active recovery.	↓ of CWI on subsequent (**@ 40 min**) cycling sprint performance.	Crampton et al
Recreationally trained subjects (13 M, 4 F, 21 Y).	Cycling: 2x 30s all-out cycling bouts with 60 min rest.	Crossover design. Immersion 10min after the 1st sprint: - CWI: 13-14°C for 15 min. - CON: 15 min PR.	Reduced peak PO and total work during the 2nd 30-s all-out cycling sprint in CWI but not CON conditions	↓ of CWI on subsequent (**@ 60 min**) cycling sprint performance.	Crowe et al.
Well trained swimmers (5 M, 5 F, 19 Y).	Swimming: - 2x 100m sprints with 30 min rest.	Crossover design. Immersion 5 min after the 1st sprint: - CWI: 14-15°C for 5 min (up to the neck). - CON: 5 min PR.	-Greater swimming time during the 2nd 100m sprint in CWI vs. CON.	↓ of CWI on subsequent (**@ 30 min**) swimming performance.	Parouty et al.
Recreationally trained subjects (8 M, 24 Y).	Running: - 12 x 120m maximal sprints with 2.5min rest.	Cross over design: - CWI: 10°C for 10 or 30 min. - CWI: 20°C for 10 or 30 min. - CON: 45 min PR.	- Similar recovery of SJ performance in CWI conditions and CON **@ 1, 2, 24 and 48 h** post-Ex. - Similar reduction of DJ performance **@ 1-2 h** post-Ex in all conditions, and full recovery of DJ performance only in 10°C-CWI (10 and 30 min).**@ 24-48 h** post-Ex	Ø of CWI on SJ performance **@ 1 h to 48 h**. ↑ of CWI (10°C) on DJ performance **@ 24-48 h**.	White et al.
Repeated post-exercise exposures					
Recreationally trained subjects (16 M, 25 Y).	6 wk- cycling training (3 sessions/wk): - 4-6 x 30s all-out bouts with 4 min rest.	Immersion after each session, 2 groups: - CWI: 10°C for 15 min. - CON: PR.	- Similar improvement of 2km-TT and 20km-TT performance, peak PO and VO2peak (incremental test) in CWI and CON groups.	Ø of CWI on endurance performance.	Broatch et al.