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

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ABSTRACT

Introduction: Cold-water immersion (CWI) has emerged as one of the most popular post-exercise recovery interventions for avid exercisers. It has been suggested that CWI can help accelerate the recovery of muscle fatigue following exercise. However, little scientific evidence exists to support such claims, especially following high-intensity interval exercise (HIIE). **Methods:** To investigate the use of CWI following HIIE, 12 young recreationally active individuals were recruited to participate in a randomized cross-over study involving repeated all-out contractions of the ankle dorsiflexor muscles followed by 10 min of room temperature rest (RT) or CWI. Following the recovery interventions, neuromuscular function, intramuscular temperature, and next-day fatigue resistance of the ankle dorsiflexor muscles were performed throughout a 24-h recovery period. **Results:** CWI had no effect on MVC torque as both conditions remained impaired for up to 3-h following exercise and recovery with full recovery at 24-h. There were no changes to VA% during exercise or at any point throughout the 24-h recovery period. CWI was shown to accelerate the recovery of the 10:50Hz torque ratio within 30 min post-CWI compared to RT, which remained impaired for up to 3-h. However, further analysis showed that the 10Hz torque remained impaired yet indifferent between the two conditions for up to 3-h, whereas the 50Hz torque remained impaired in the CWI condition for up to 3-h compared to RT, which displayed immediate recovery. No difference in next day HIIE performance and fatigue resistance was seen between the two conditions for peak isokinetic velocity or total contractions per set. **Conclusion:** Although the recovery of 10:50Hz torque was accelerated following CWI, it was at the expense of depressed 50Hz maximal tetanic force, which was shown to remain impaired for considerably longer than RT. Furthermore, MVC torque impairment and recovery were similar between the two conditions whereas VA% was not affected at any point during exercise or during the post-exercise recovery period. Lastly, there was no difference in next day performance between the recovery conditions. Altogether, this suggests that CWI offers no substantial benefit as an effective post-exercise recovery modality following HIIE.

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LIST OF ABBREVIATIONS

ATP: Adenosine triphosphate

Ca^{2+}: Calcium

CWI: Cold-water immersion

HIE: High intensity exercise

mTOR: Mammalian target of rapamycin

MVC: Maximal voluntary contraction

PLFFD: Prolonged low-frequency force depression

ROS/RONS: Reactive oxygen and nitrogen species

RT: Room temperature

SERCA: Sarcoplasmic reticulum calcium ATPase

SIE: Sprint interval exercise

SR: Sarcoplasm reticulum

VA%: Voluntary activation

CHAPTER 1:

LITERATURE REVIEW

1.1 Introduction to cold water immersion as an exercise-dependent recovery modality

1.1.1 Overview of post-exercise cooling

Post-exercise recovery modalities aimed at accelerating the recovery of muscle fatigue and damage have long been sought after and prioritized by athletes and coaches. One such recovery modality has been the use of cold-water immersion (CWI), mainly for its simplistic and cost-effective approach and application (Batista et al., 2023; Machado et al., 2016; Moore et al., 2022). Protocols of CWI vary, but CWI is generally considered to be partial or complete immersion of the body in water temperatures of ≤ 15 ºC (Machado et al., 2016; Malta et al., 2021; Petersen & Fyfe, 2021; Sánchez-Ureña et al., 2015). Post-exercise CWI is proposed to aid in managing many exercise-induced responses, such as inflammation and perceived muscle soreness and/or pain (Bouzid et al., 2018; Chaillou et al., 2022; Choo et al., 2022; Malta et al., 2021; Petersen & Fyfe, 2021; Sánchez-Ureña et al., 2015). However, the exact mechanisms for the proposed benefits of CWI are not entirely certain. It seems that the hydrostatic effects of CWI may result in changes to local blood circulation and fluid shifts (Batista et al., 2023; Bouzid et al., 2018; Hyldahl & Peake, 2020; Malta et al., 2021; Moore et al., 2022; Petersen & Fyfe, 2021; Sánchez-Ureña et al., 2015; Tabben et al., 2018; Xiao et al., 2023). However, types of exercise contribute to muscle fatigue differently and elicit different post-exercise adaptive responses, suggesting that post-exercise CWI effectiveness should be considered task-dependent (Chaillou et al., 2022; Malta et al., 2021).

Fatigue can be broken down into central and peripheral fatigue. Central fatigue is considered a progressive exercise-induced degradation in voluntary activation due to decreased motor neuron excitation (Boyas & Guével, 2011), whereas peripheral fatigue occurs distal to the neuromuscular junction and is influenced by metabolic and biochemical changes within the muscle (Tornero-Aguilera et al., 2022). A study by Edwards et al. (1977) showed for the first time the

delayed and slow recovery of low-frequency force compared to high-frequency force, suggesting that peripheral fatigue, referred to as prolonged low-frequency force depression (PLFFD), can significantly impair and affect subsequent performance if not recovered (Fig. 1). High-frequency contractions are considered to be equivalent to the activation of muscle during all-out maximal efforts, such as power lifting or in brief sprinting efforts. In contrast, low-frequency contractions are considered equivalent to the activation of muscle during activities involving submaximal effort contractions, such as walking or jogging. When considering low-frequency recovery, the recovery of submaximal contractions is paramount to sports incorporating both maximal and submaximal efforts. Furthermore, if not recovered sufficiently, PLFFD may persist and further exacerbate symptoms of fatigue and overtraining (Cheng et al., 2020b). As seen with many types of exercise, most notably endurance, resistance, and sprint exercise, the presence of peripheral fatigue caused by different exercise-induced responses can significantly impair performance (Aibara et al., 2020; Balnave & Allen, 1995; Cheng et al., 2017; Ørtenblad et al., 2022; Kamandulis et al., 2022; Skurvydas et al., 2016), indicating that post-exercise strategies aimed at accelerating the recovery of muscle fatigue, for both maximal and submaximal contractions, is critically important, especially for athletes performing bouts of exercise with limited rest in-between.

It has been previously shown that reduced intramuscular temperatures can have dramatic effects on isometric force, maximal rates of force development, and speed of muscle relaxation (de Ruiter et al., 1999). In addition, it has also been shown that concentric contractions are more affected with reduced intramuscular temperatures than eccentric contractions (de Ruiter & Haan, 2001), highlighting the different effects that CWI has on muscle performance. Furthermore, when considering the recovery of low- and high-frequency contractions following exercise (Fig. 1), de Ruiter et al. (1999) showed that different muscle temperatures can impact the force-frequency

curve. Specifically, reduced intramuscular temperatures have the potential to increase force at low frequencies, whereas at higher frequencies, reduced intramuscular temperature is shown to reduce force output (de Ruiter et al., 1999). Therein lies the importance of assessing muscle recovery using both low- and high-frequency contractions as both are affected differently following exercise and cooling.

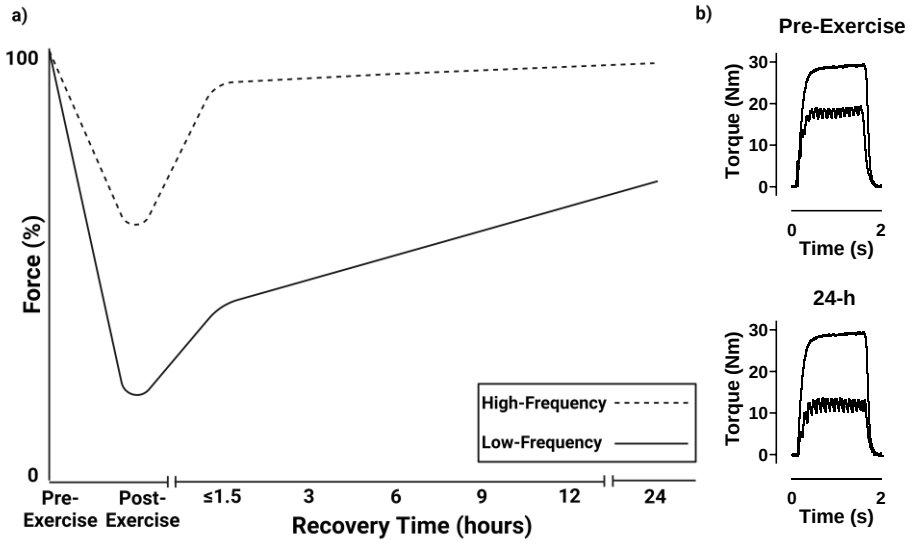

Figure 1: Schematic representation of the recovery of low- and high-frequency force after fatigue. a) The impaired recovery of low-frequency force following metabolically demanding exercise compared to high-frequency force. **b)** Impaired electrically evoked low-frequency force 24-h later compared to full recovery of forces at high frequencies.

This review will explore some of the similarities and differences between three types of exercise – endurance, resistance, and sprint – and how prescribing post-exercise CWI might affect recovery. Noteworthy, very few studies investigate skeletal muscle fatigue from a physiological perspective in conjunction with post-exercise CWI. Specifically, assessments of skeletal muscle fatigue did not include both low- and high-frequency contractions. The studies mentioned in this literature review performed only maximal effort contractions to assess the fatigue and recovery of muscle function, which limits the results attempting to investigate skeletal muscle recovery following exercise. Nonetheless, this review will investigate post-exercise performance variables,

muscle damage markers, and perceived recovery and/or soreness following CWI to determine muscle recovery status. Furthermore, specific mechanisms associated with cooling for each exercise type will be investigated, keeping in mind that most of the suggested mechanisms are not entirely known and are purely speculative, thus providing future avenues for exploration. In addition, specific cutoffs of acute (≤ 1 hour) versus later (≥ 1 hour) recovery time points are used to differentiate the acute effects of cooling (i.e., when the effects of CWI are suggested to be most prominent) and the later effects (when physiological responses such as tissue temperature and blood flow would most likely return to baseline).

1.1.2 Effects of cooling following endurance exercise

Endurance exercise is generally defined as aerobic exercise consisting of prolonged bouts at submaximal intensities to stimulate cardiovascular, respiratory, and skeletal muscle adaptations (Chaillou et al., 2022). Furthermore, fatigue and impaired endurance performance can result from a multitude of physiological factors, such as metabolite accumulation, hyperthermia, dehydration, and glycogen depletion (Chaillou et al., 2022). It has been suggested that CWI performed following endurance exercise improves subsequent performance and fatigue resistance if subsequent performance occurs ≤ 1 hour later (Chaillou et al., 2022; Choo et al., 2022; Crampton et al., 2013; Dunne et al., 2013; Malta et al., 2021; McCarthy et al., 2016; Moore et al., 2022; Peiffer et al., 2010; Vaile et al., 2008; Yeargin et al., 2006). In the study by Crampton et al. (2013), it was shown that CWI led to improved submaximal endurance cycling compared to other popular recovery strategies, such as active recovery and contrast water therapy, which involves alternating between hot and cold exposure every few minutes for 10-30 minutes (Chaillou et al., 2022; Hyldahl & Peake, 2020; Sánchez-Ureña et al., 2015).

When considering the later stages of recovery (≥ 1 hour), some evidence suggests that CWI does not affect subsequent performance and recovery (Anderson et al., 2018; Broatch et al., 2017; Chaillou et al., 2022; Choo et al., 2022; Malta et al., 2021; Petersen & Fyfe, 2021) whereas others suggest potential impairments to performance (Cheng et al., 2017). The study by Cheng et al. (2017) showed that CWI has the potential to impair subsequent endurance bouts due to the effects of cooling slowing glycogen resynthesis rates. This has significant implications for endurance athletes as glycogen resynthesis is a slow process (Cheng et al., 2020a; Murray & Rosenbloom, 2018), and potentially delaying glycogen resynthesis in the immediate hours following exercise might be counterproductive to recovery.

To summarize, it seems that acute CWI performed prior to subsequent exercise (≤ 1 hour) seems to indicate favourable results. However, when considering the later stage of recovery and performance (≥ 1 hour), it seems that CWI could impair performance more than enhance it. Thus, endurance athletes should take caution and weigh the risks versus rewards when performing post-exercise CWI as a recovery intervention.

1.1.3 Proposed mechanisms of CWI following endurance exercise

It has been suggested that the early benefits of CWI on endurance performance could be from a reduction in local peripheral blood flow, thus increasing central blood flow, leading to an increase in stroke volume and cardiac output (Chaillou et al., 2022; Choo et al., 2022; Moore et al., 2022; Sánchez-Ureña et al., 2015). In addition, the benefits of CWI and subsequent improvement in endurance performance might stem from reducing cardiovascular strain (Chaillou et al., 2022), indicated by decreases in body temperature and heart rate following the acute recovery of CWI (Choo et al., 2022). Furthermore, lower body temperatures have been associated with reducing nerve conduction velocity and mechanoreceptor activity, thus blunting the stretch-

reflex response and inhibiting potential muscle spasms associated with perceived soreness and/or pain (Bouzid et al., 2018). The sensations of pain following cooling are also suggested to be reduced due to local vasoconstriction reducing lymphatic and blood vessel permeability, thus decreasing the interstitial space and potentially reducing inflammation and edema (Sánchez-Ureña et al., 2015).

However, as shown by Cheng et al. (2017), muscle cooling can impair glycogen resynthesis, which was shown to reduce subsequent performance output. The importance of glycogen, especially during endurance events, is that it is the primary fuel source supplying adenosine triphosphate (ATP) availability (Hargreaves & Spriet, 2020). Reduced glycogen resynthesis rates can affect force generation, as it was also shown to affect the recovery of low-frequency force and fatigue resistance, which was related to a decrease in sarcoplasmic reticulum (SR) calcium (Ca^{2+}) release (Cheng et al., 2017).

1.1.4 Effects of cooling following resistance exercise

Resistance exercise is generally considered high-intensity skeletal muscle exercise at or near one repetition maximum load (Chaillou et al., 2022), aimed at increasing skeletal muscle mass and improving the rate and/or capacity of force generation (Petersen & Fyfe, 2021). Furthermore, resistance exercise is associated with myofibrillar damage, leading to muscle fatigue (Cheng et al., 2017). The proposed theory for implementing CWI following resistance exercise might be due to the potential improvements in perceived muscle soreness, muscle damage, and fatigue (Petersen & Fyfe, 2021). The consensus of post-resistance training CWI varies, with some showing moderately positive acute effects on strength (Chaillou et al., 2022; Roberts et al., 2015a) and others showing detrimental long-term effects on strength and hypertrophy (Hyldahl & Peake, 2020; Malta et al., 2021; Roberts et al., 2015b). Regarding the acute effects of CWI following

resistance exercise (≤ 1 hour), the study by Roberts et al. (2015a) was able to show that CWI better preserved maximal voluntary contraction force. However, Chaillou et al. (2022) suggested that any strength improvements following CWI are modest at best.

When considering the later stages of recovery (≥ 1 hour), it seems that CWI does not affect subsequent performance outcomes (Chaillou et al., 2022). In the review by Chaillou et al. (2022), performance outcomes such as maximal knee extensor torque production, jump performance, and total knee extensor work performed hours later were no different from the control conditions. This might stem from the modest benefits of strength preservation with CWI having worn off as tissue temperatures return to pre-exercise levels. However, acute changes in skeletal muscle temperature have been shown to have drastic long-term effects on strength and hypertrophy (Hyldahl & Peake, 2020; Malta et al., 2021; Roberts et al., 2015b). Specifically, Malta et al. (2021) suggested that chronic use of CWI reduces training-induced increases in type II muscle fibre cross-sectional area, the number of myonuclei per fibre, and anabolic signalling, possibly due to the vasoconstrictive effects of local cooling.

To summarize, in the acute recovery phase (≤ 1 hour) following resistance exercise and CWI, modest improvements in strength are seen compared to other popular forms of post-exercise recovery, such as active recovery. However, when investigating the later recovery phase (≥ 1 hour), it seems CWI does not affect subsequent exercise performance and may drastically affect overall strength and hypertrophy development. Thus, athletes engaging in resistance exercise should refrain from employing CWI as a post-exercise recovery intervention when trying to maximize strength and/or hypertrophy.

1.1.5 Proposed mechanisms of CWI following resistance exercise

Improvements in acute strength following the initial resistance bout and CWI are suggested to result from reduced pain perception (Chaillou et al., 2022). It is suggested that CWI may inhibit metabolite-sensitive III and IV muscle afferents, thus reducing pain sensations (Chaillou et al., 2022). Furthermore, acute increases in strength may be due to local cooling enhancing low-frequency force caused by the slowing of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps (Chaillou et al., 2022), thus favouring strength endurance preservation. In addition, local muscle cooling has been shown to increase muscle-tendinous stiffness (Mallette et al., 2021), which may explain why dynamic force might be improved immediately following CWI (Moore et al., 2022). However, contrasting views exist regarding dynamic strength improvements following CWI, as Petersen & Fyfe (2021) have suggested impaired dynamic strength following CWI. Thus, when considering the potential acute effects of cooling on dynamic strength, the rate of which force is produced is a critical factor as increased rates of force development are negatively affected with CWI application (Petersen & Fyfe, 2021).

When considering the long-term effects of CWI on resistance training adaptations, CWI may be more detrimental than beneficial (Petersen & Fyfe, 2021; Roberts et al., 2015b). It was shown by Roberts et al. (2015b) that 12 weeks of resistance training followed by CWI after each training bout led to reductions in muscle mass and strength. In addition, when looking at the acute anabolic responses following CWI, the activation of satellite cells and specific kinases involved in the mammalian target of rapamycin (mTOR) pathway were shown to be delayed or suppressed (Malta et al., 2021; Roberts et al., 2015b), consistent with the long-term impairments seen with routine cooling on muscle mass and strength development. Furthermore, heat shock proteins are critical regulators for cellular homeostasis and protein maintenance (Petersen & Fyfe, 2021). It has

been shown that CWI may alter heat shock protein regulation and further contribute to the impaired adaptive responses seen with regular CWI application (Petersen & Fyfe, 2021).

When considering the post-exercise regimens following exercise, specifically resistance exercise, protein consumption is common to meet protein synthetic demands. Malta et al. (2021) showed that protein ingestion following CWI led to decreased myofibrillar protein synthesis, suggested to be due to a lack of amino acid transport and delivery to the muscle from the vasoconstrictive effect and reduced local blood flow as a result of muscle cooling. Overall, it seems that CWI for resistance training athletes seems more detrimental to the physiological adaptations associated with resistance exercise. Thus, athletes should refrain from CWI as a post-exercise resistance training intervention, even considering the modest acute benefits of strength preservation with local cooling.

1.1.6 Effects of cooling following sprint exercise

Sprint exercise is generally defined as maximal effort sprints involving anaerobic metabolism for very short periods to develop power output and anaerobic capacity (Chaillou et al., 2022). Sprint exercise has been shown to result in the significant accumulation of specific metabolites like lactate, hydrogen and inorganic phosphate ions, and reductions in cytosolic ATP (Karatzaferi et al., 2001; Place et al., 2015; Westerblad et al., 2010; White et al., 2014; Yanagisawa et al., 2014), all of which to some extent contribute to either acute fatigue or PLFFD (Cheng et al., 2020a; Place et al., 2015). During the acute recovery phase following CWI (≤ 1 hour), it has been shown that subsequent performance is impaired, demonstrated through increased sprint repeatability times (Chaillou et al., 2022; Parouty et al., 2010; Tabben et al., 2018) and reduced power output (Choo et al., 2022; Crampton et al., 2014). In the study by Parouty et al. (2010), swimmers exposed to whole-body CWI had increased swimming sprint times 30 minutes

following their initial bout. The study by Crampton et al. (2014) showed that peak and mean cycling power output was reduced 30 minutes after exposure to lower-body CWI.

When considering later time points (≥ 1 hour), it seems that CWI does not affect subsequent exercise performance (Chaillou et al., 2022; Hyldahl & Peake, 2020; Jakeman et al., 2009; Leeder et al., 2019; Tabben et al., 2018; White et al., 2014). In the study by Tabben et al. (2018), CWI did not affect sprint performance 24 hours following the initial bout. Similarly, the study by White et al. (2014) showed no effect on maximal force generation in the hours following (1-2 hours) the initial bout of maximal sprints and CWI. However, in that same study by White et al. (2014), it was also shown that ≥ 24 hours following the initial bout of sprinting and CWI, drop jump performance had recovered to a greater extent compared to the control condition, whereas measures of squat jump performance and maximal voluntary contractions remained indifferent between the two conditions. In addition, the study by Leeder et al. (2019) showed that CWI following simulated sprint field sports better-preserved sprint times but not muscle power, measured through counter-movement jumps, or strength measured through maximal voluntary contractions. What complicates CWI following sprint exercise is the limited number of studies investigating CWI and sprint exercise, especially much later time points (≥ 24 hours) of repeated sprint exercise (Hyldahl & Peake, 2020), leading to inconsistent and inconclusive recommendations for CWI recovery in sprint-like sports.

To summarize, from the available studies investigating post-exercise CWI following sprint exercise, it seems that CWI is ineffective and detrimental to subsequent performance if performed ≤ 1 hour later. Furthermore, when considering the later stages of recovery and performance (≥ 1 hour), inconsistencies seem to exist within the literature, with some showing minimal benefits on some performance measures, whereas others show no difference. However, the limited number of

studies prioritizing later recovery performance measures should be noted. Therefore, future studies should aim to investigate later time points of recovery following CWI and sprint exercise to determine how CWI affects muscle recovery.

1.1.7 Proposed mechanisms of CWI following sprint exercise

Sprint exercise produces metabolic substrates such as reactive oxygen species (ROS), which contribute to impaired Ca^{2+} release and sensitivity, leading to muscle fatigue and the cessation of exercise (Cheng et al., 2020a; Cheng et al., 2017). It has been suggested that CWI might help limit ROS generation due to the vasoconstrictive effects regulating hypoxic stress (Klich et al., 2018) and better preserve force-generating capacity as increased ROS has been shown to impair excitation-contraction coupling processes (Cheng et al., 2020a; Place et al., 2015). However, accelerating the recovery and removal of ROS, as suggested with local cooling, may not be as beneficial as initially thought, as the natural clearance of ROS beneficially contributes to exercise-induced muscle adaptations (Chaillou et al., 2022). Therefore, post-exercise CWI should be used sparingly, such as when rapid recovery and clearance of ROS are needed for subsequent exercise.

Sprint exercise involves maximal effort dynamic contractions, and CWI has been shown to delay and slow action potential generation and neural drive (Chaillou et al., 2022) as well as cross-bridge cycling (Tabben et al., 2018), which may explain the performance impairments seen during the acute recovery phase following CWI (≥ 1 hour). Furthermore, CWI has been shown to reduce the rate of force development both voluntarily and involuntarily (Choo et al., 2022), thus limiting maximal dynamic effort and force-generating capacity. Therefore, if employing CWI as a post-exercise recovery strategy for subsequent sprint exercise, enough time should be devoted to

re-warming the muscles for exercise and allowing for optimal movement and performance output (Tabben et al., 2018).

Favourable improvements in perceived recovery might be due to CWI enhancing parasympathetic activity (Chaillou et al., 2022; Zhang et al., 2022) and altering the cerebral dopaminergic and noradrenergic systems which have been associated with fatigue development (Tabben et al., 2018). Although beneficial in theory, for sprint athletes, the withdrawal of sympathetic activity can drastically reduce performance due to parasympathetic activity reducing heart rate acceleration and limiting cardiac output (Chaillou et al., 2022). Therefore, similar to the above recommendations of CWI for sprint athletes, employing CWI should be carefully thought out. Malta et al. (2021) suggested that CWI might be training block-specific and not implemented if prioritizing power, strength, or hypertrophy.

1.2 Overall conclusion

This review investigated three exercise types and how CWI might affect recovery differently. For endurance athletes in the acute recovery phase (≤ 1 hour), CWI might help with subsequent performance, but it may not affect the later stages of recovery (≥ 1 hour). However, endurance athletes should also consider the amount of glycogen repletion needed for subsequent exercise (Cheng et al., 2017). When looking at CWI following resistance exercise, modest improvements in strength are seen in the acute recovery phase (≤ 1 hour), whereas no effects are seen in the later stages (≥ 1 hour). However, caution should be taken when implementing long-term CWI following resistance training sessions, as cooling has been shown to suppress anabolic signalling necessary for strength and/or hypertrophy gains (Malta et al., 2021; Petersen & Fyfe, 2021; Roberts et al., 2015b). Lastly, there are only a few studies that have explored the use of CWI following sprint exercise, but from this limited investigation they showed detrimental effects in

the acute phase of recovery (≤ 1 hour) and no effects in the later stages (≥ 1 hour) (Hyldahl & Peake, 2020). Such differing outcomes with CWI and exercise suggest that CWI should be used sparingly, and consideration should be given when weighing its risks versus rewards. This review highlights what Chaillou et al. (2022) and Malta et al. (2021) have suggested: CWI should be considered an exercise-dependent recovery modality.

Regarding CWI and exercise type, this review indicates that both endurance and resistance exercise have been well explored regarding potential performance-related effects, whereas sprint exercise has not. Sprint exercise represents an increasingly popular exercise type due to its time-efficient benefits on exercise-induced responses (MacInnis & Gibala, 2017). Thus, future work should aim to elucidate the inconsistencies that exist with CWI application following sprint exercise. Furthermore, the use of submaximal contractions to assess muscle recovery are necessary as studies investigating local cooling effects on muscle recovery following sprint exercise have used maximal effort contractions. As shown previously, submaximal contractions, termed low-frequency contractions, take much longer to recover than high-frequency contractions. Therefore, to best represent the recovery of skeletal muscle following fatiguing exercise, investigating both low- and high-frequency contractions is necessary. In addition, assessing repeated sprint exercise performance is beneficial to sprint athletes as multi-day track and field competitions require maximal effort performance with limited recovery times, suggesting that assessing next-day performance (24 hours) might be beneficial to truly elucidate whether CWI affects muscle recovery and performance outcomes.

CHAPTER 2:

RATIONALE AND HYPOTHESIS

2.1 Rationale

Sprint interval exercise has been shown to result in central and peripheral fatigue (Fernandez-del-Olmo et al., 2013; Place et al., 2015) and increased amounts of ROS/RONS generation (Sakellariou et al., 2013), which has been suggested to be a significant cause of PLFFD (Cheng et al., 2016). The implications of PLFFD have drastic effects on exercise performance as low-frequency force has been shown to be suppressed to a much greater magnitude and duration compared to high-frequency force (Edwards et al., 1977). Furthermore, reducing muscle tissue temperature has been shown to decrease ROS generation and improve force production (Klich et al., 2018; Van Der Poel et al., 2008). Therefore, post-exercise cooling might help reduce the generation of ROS and accelerate the recovery of force production within the first 3 hours of the recovery period. In addition, the acute physiological changes that cooling might cause could have implications on performance outcomes, especially 24 hours later. Sprint athletes are tasked with competing in multi-day competitions that require peak performance within 24 hours of performing the last bout. Thus, assessing muscle recovery and performance within the 24 hours following CWI will help to elucidate its effectiveness for sprint athletes. Furthermore, many, if not most, CWI-related exercise studies have used maximal effort contractions and/or perceived recovery as an outcome assessing muscle recovery. However, many psychological variables contribute to the perception of readiness and/or recovery, suggesting that perceived variables are not the best indicator of physiological muscle recovery. Furthermore, maximal effort contractions are less sensitive indicators of physiological fatigue, as submaximal contractions have been shown to remain impaired for considerably longer (Cheng et al., 2020a; Edwards et al., 1977; Place et al., 2015).

Thus, this proposed study will determine the effects of CWI on the recovery of skeletal muscle force generation and its contributions to central and/or peripheral fatigue and sprint repeatability following SIE.

2.2 Hypothesis

It is hypothesized that CWI will be beneficial in the acute recovery (≤ 3 hours) of force generation from the effects of cold-water reducing muscle temperature, resulting in lowered oxidative stress compared to room temperature recovery. Additionally, it is hypothesized that CWI will not affect the recovery of force generation or performance 24 hours after the initial bout of exercise as it is proposed that the acute effects and physiological changes seen with cooling will have returned to baseline within a few hours following immersion.

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CHAPTER 3:

MANUSCRIPT

3.1 Introduction

In recent years, the popularity of sprint exercise, a sub-form of high-intensity exercise (HIE), has gained increasingly popular due to its time-efficient way of inducing similar adaptions to that of traditionally used continuous aerobic exercise (Chaillou et al., 2022; MacInnis & Gibala, 2017). Sprint interval exercise (SIE), a form of HIE, involves repeated bouts of brief all-out exercise with short rest periods between each bout (Chaillou et al., 2022; Wingo et al., 2018). Due to the strenuous nature and high metabolic demand of SIE, recent studies have shown fatigue-induced reductions in muscle contractile force in relation to the onset of neuromuscular fatigue (Fernandez-del-Olmo et al., 2013; Place et al., 2015). Neuromuscular fatigue can be broken down into central and peripheral fatigue, in which the type of exercise performed can contribute to the extent of fatigue experienced (Gandevia, 2001). Central fatigue is considered a progressive exercise-induced degradation in voluntary activation due to decreased motor neuron excitation (Boyas & Guével, 2011), whereas peripheral fatigue occurs distal to the neuromuscular junction and is influenced by metabolic and biochemical changes within the muscle (Tornero-Aguilera et al., 2022). A study by Edwards et al. (1977) showed for the first time the delayed and slow recovery of low-frequency force compared to high-frequency force in human skeletal muscle, suggesting that peripheral fatigue, better known as prolonged low-frequency force depression (PLFFD) can persist for hours if not days following fatigue-inducing exercise. This can have drastic effects on subsequent exercise performance as PLFFD has been linked to prolonged symptoms of fatigue and overtraining if not recovered sufficiently (Cheng et al., 2020b).

A study by Place et al. (2015) showed that reactive oxygen and nitrogen species (ROS/RONS) generation is markedly increased during HIE, suggesting that ROS/RONS production is a primary exercise-induced response leading to PLFFD. Furthermore, Fernandez-

del-Olmo et al. (2013) showed that just one bout of 30-second all-out cycling exercise can markedly reduce low-frequency force long after the initial bout, suggesting that ROS/RONS generation is a rapidly occurring process with prolonged effects on performance. PLFFD is suggested to be due to the delayed and slow recovery of sarcoplasmic reticulum (SR) calcium (Ca^{2+}) release regulating muscle contractions (Aibara et al., 2020). Furthermore, treatments available to limit ROS/RONS generation include antioxidant treatment. However, as noted by Cheng et al. (2020a), antioxidant treatments shift the cause of PLFFD from impaired Ca^{2+} release to reduced myofibrillar Ca^{2+} sensitivity, but without fixing the impaired muscle force generation. Therefore, effective post-exercise recovery modalities for athletes are necessary to limit the generation and accelerate the recovery of ROS/RONS following fatiguing HIE. One such recovery modality that has gained increasingly popular is the use of cold-water immersion (CWI), which has been suggested to limit ROS/RONS generation and damage (Klich et al., 2018; White & Wells, 2013) by reducing intramuscular temperature (Van Der Poel et al., 2008).

The use of CWI is one of the most widely used post-exercise recovery interventions due to its cost-effective and time-efficient application (Batista et al., 2023; Machado et al., 2016; Moore et al., 2022). The application of CWI is proposed to aid in managing many exercise-induced responses such as inflammation and perceived muscle soreness and/or pain (Chaillou et al., 2022; Choo et al., 2022; White & Wells, 2013) due to the hydrostatic effects of CWI resulting in changes to local blood circulation and fluid shifts (Batista et al., 2023; Bouzid et al., 2018; Hyldahl & Peake, 2020; Malta et al., 2021; Moore et al., 2022; Petersen & Fyfe, 2021; Sánchez-Ureña et al., 2015; Tabben et al., 2018; Xiao et al., 2023). Although popular, the effectiveness and known mechanisms of CWI remain elusive and speculative at best, especially following sprint-specific sports (Hyldahl & Peake, 2020). Furthermore, when considering prolonged recovery times and

subsequent assessments of skeletal muscle recovery, inconsistencies on performance-related outcomes are seen due to the limited number of studies investigating CWI and HIE (Leeder et al., 2019; Tabben et al., 2018; White et al., 2014) compared to other exercise types like endurance and resistance exercise (Chaillou et al., 2022; Choo et al., 2022; Hyldahl & Peake, 2020). Of the available studies investigating CWI and HIE, maximal effort performance measures such as repeated sprint performance and power output were used to assess muscle recovery. However, maximal effort contractions have been shown to be less sensitive indicators of physiological muscle fatigue, as submaximal contractions have been shown to remain impaired for considerably longer, indicated by the presence of PLFFD following fatigue-inducing exercise (Cheng et al., 2020a; Edwards et al., 1977; Place et al., 2015). Therefore, to measure skeletal muscle recovery and force-generating capacity, the use of both maximal effort contractions and submaximal contractions is necessary.

Thus, the aim of this study is to investigate the effects of CWI on the recovery of skeletal muscle force generation and its contributions to central and/or peripheral fatigue and sprint repeatability following high-intensity SIE. It is hypothesized that CWI will be beneficial in accelerating the acute recovery (≤ 3-h) of force generation due to lowered intramuscular temperature. Additionally, it is hypothesized that CWI will not affect the recovery of force generation or performance 24 hours after the initial bout of exercise as it is proposed that the acute effects and physiological changes seen with cooling will have returned to baseline within a few hours following immersion.

3.2 Materials and methods

3.2.1 Participants

Twelve healthy participants (10 M, 2 F) were recruited to participate in this randomized cross-over study. The participants were 23.3 ± 3.1 years of age with an average height of 176.6 ± 8.1 cm and a mass of 84.1 ± 13.5 kg. Participants were recreationally active and not participating in organized sport at the time of testing. Participants were free of acute or chronic disease affecting their neuromuscular performance. All participants gave informed, written consent and the study was approved by the local ethics committee on human research (York University Human Participants Ethics Review Committee) and conformed to the Canadian Tri-Council Research Ethics Guidelines.

3.2.2 Experimental overview

Experiments consisted of 5-6 laboratory visits. The first visit was a familiarization trial for participants to gain an understanding of the experimental protocol and habituate themselves to the equipment. If necessary, a second familiarization trial was performed to ensure the participant understood the procedures and could achieve > 90% of voluntary activation (VA%). On experimental days, participants were told to refrain from caffeine consumption on the day of and strenuous exercise 24 hours before their laboratory visit. Upon arriving at the laboratory, baseline neuromuscular assessments and intramuscular temperature measures of the lower left leg were recorded (Fig. 1). From there, participants performed sprint interval dorsiflexion exercise in the lower left leg. Immediately following the exercise, and in a randomized order, the lower left leg was placed in either cold water (CWI) at 10º C for 10 minutes or kept at room temperature (RT) for 10 minutes (Fig. 1). The immersion temperature of 10º C and duration of 10 minutes was selected as it is one of the most commonly used immersion protocols within the literature (Bouzid

et al., 2018; Jakeman et al., 2009; Roberts et al., 2015; Zhang et al., 2022) and fits within the range at which CWI is suggested to be most beneficial and tolerable by individuals (Batista et al., 2023; Machado et al., 2016; Peiffer et al., 2010; Petersen & Fyfe, 2021; Zhang et al., 2022). During the post-exercise recovery time period, neuromuscular assessments were performed at 0 minutes, 30 minutes, 1 hour, 3 hours, and 24 hours later (the 24-h time was laboratory visit 4) (Fig. 1). In addition, the intramuscular temperature of the exercised leg was recorded regularly for the 3 hours following the post-exercise recovery condition. Furthermore, 24 hours after the initial bout of exercise, a second sprint interval exercise bout was performed in the same leg. Visits 5 and 6 were identical to visits 3 and 4, with the only difference being the recovery intervention (i.e., either CWI or RT), and took place within the following 2 weeks allowing for adequate rest and recovery to ensure performance was not affected from the initial condition.

3.2.3 Experimental set-up

A HUMAC NORM isokinetic dynamometer (CSMi Medical Solutions, Stoughton, MA, USA) was used throughout the study to perform torque and position values for neuromuscular assessments and exercise performance. Participants were seated with their left hip at 110º of flexion and knee at 120º of extension. Any movement from the torso was restricted with a 4-point seatbelt harness. The left foot was fixed to a dorsiflexor dynamometer foot pedal with a nonelastic tightening strap positioned over the ankle and two others strapped at the mid-portion of the foot. The maximal ankle dorsiflexion angle was set to 130º to allow for a 40º joint range of motion to optimize plantar flexion stretch to ensure a proper range of motion during the exercise. During the neuromuscular assessments, the ankle dorsiflexion angle was fixed at 130º as pilot testing data indicated that peak torque was achieved at that joint angle.

3.2.4 Baseline voluntary and involuntary neuromuscular assessment

A clinical bar stimulating electrode (model E.SB010, Digitimer North America, Fort Lauderdale, FL, USA) was used to stimulate the peroneal nerve resulting in electrically evoked contractions of the primary ankle dorsiflexor muscle, the tibialis anterior. The peroneal nerve was found by locating the fibular head and moving both inferiorly and posteriorly. Peripheral nerves were evoked by a single pulse (single 200-μs square wave pulses, 400 V) from a constant high-current voltage stimulator (model DS7R; Digitimer, Welwyn Garden City, UK). The current was gradually increased by 5mA until a plateau in twitch force occurred. The current used for the single pulse was the current used for the low-frequency (10Hz) and high-frequency (50Hz) involuntary isometric tetanic stimulations. To determine the VA% current using the interpolated twitch technique, the single pulse current was superimposed by 30% of that value to determine the doublet current (100Hz). Superimposing the current by 30% was done to achieve maximum twitch force (Cheng & Rice, 2009) in relation to MVC force (Gandevia, 2001). During the interpolated twitch technique, participants were verbally encouraged to contract as forcefully as possible during the maximal voluntary isometric contraction which was immediately followed by a potentiated resting doublet stimulation. The VA% was calculated using the following equation: VA% = (1 – interpolated twitch torque/resting twitch torque) x 100 (Gandevia, 2001). To assess peripheral fatigue, the peak torque achieved during the 10Hz and 50Hz tetanic stimulations were obtained, respectively, and a ratio of the 10 to 50Hz torque was calculated. This ratio is used to determine fatigue-related changes in SR Ca^{2+} regulation as shown by the sigmoidal curve showing low- and high-frequency force and Ca^{2+} release, where greater impairments to force are seen at low versus high frequencies (Cheng et al., 2017; Cheng et al., 2018).

3.2.5 Intramuscular temperature

Before installing the intramuscular probe, the muscle belly of the tibialis anterior was located as the halfway point of the lower left leg and moving laterally from the tibia. Upon cleaning the area with an alcohol wipe, an autoclaved tissue implantable thermocouple microprobe (model IT-21; Physitemp, Clifton, NJ, USA) was inserted roughly 2 cm into the muscle using a 21-gauge catheter. Once inserted, the microprobe plug was connected to a temperature recorder (model BAT-12; Physitemp, Clifton, NJ, USA), outputting intramuscular temperature recordings. To ensure the microprobe stayed in place with minimal movement, medical tape and athletic pro-wrap was tightly secured around the microprobe insertion site to ensure the wire stayed at the depth at which it was inserted initially.

3.2.6 Exercise

Exercise involved left-legged isokinetic contractions of the dorsiflexor muscles set at 160º/sec. The interval nature of the exercise involved 6 sets of 30-second all-out contractions with three minutes of rest between sets. This exercise protocol was selected as Place et al. (2015) showed that 6 sets of 30-second all-out cycling markedly increased the production of ROS/RONS resulting in PLFFD and skeletal muscle fatigue. Furthermore, the isokinetic dynamometer was set up to involve only concentric contractions, thus isolating ROS/RONS related impairments and not eccentric specific myofibrillar protein damage seen following exercise which can also lead to PLFFD (Place et al., 2015). During the exercise, participants received verbal encouragement from the experimenter and visual feedback of contraction torque and range of motion on a second monitor. During the initial bout of SIE, neuromuscular assessments were performed immediately after each set to determine the extent of fatigue during exercise. The second SIE bout, performed 24 hours later, was identical to the initial bout.

3.2.7 Recovery protocol

Immediately following exercise, participants performed the post-exercise recovery. Before immersing the exercised leg in cold water, the lower left leg was placed in a bag to prevent direct skin contact and potential discomfort from the cold water. The CWI condition involved complete immersion of the lower left leg (up to the tibial tuberosity) in cold water and crushed ice at 10º C for 10 minutes. Water temperature was monitored using a digital thermometer, and crushed ice was added as needed to maintain the water temperature at 10º C. For the RT condition, participants were asked to rest seated at room temperature for 10 minutes.

3.2.8 Statistical analysis

Two-way repeated measures ANOVA, a mixed-effects model for repeated measures, and paired t-tests were performed as appropriate using GraphPad software (*Prism* 10.2.1, San Diego, CA, USA). Two-way ANOVA was used to compare intramuscular temperature changes between the two conditions during the post-recovery intervention across time. A mixed-effects model was used to compare neuromuscular and performance related differences between the two groups. For neuromuscular analyses using a mixed-effects model, individual data points were excluded if they resided outside a 0.95 confidence interval. A mixed-effects model was used to compare fatigue resistance (i.e., peak velocity per contraction) in all bouts of exercise per set as missing values occurred in participants that fell below the group mean for total contractions during each exercise set. Paired t-tests were performed to compare baselines measures of the two groups. In addition, paired t-tests were performed to compare mean peak velocity and mean contractions between groups. For the post hoc analysis, a Tukey's test was performed where appropriate. Data within the text, figures, and tables are represented as relative or absolute means ± SD. Statistically significant differences were determined at $p < 0.05$.

3.3 Results

3.3.1 Baseline measurements

There were no significant differences in baseline variables between the two conditions (see Table 1).

3.3.2 Neuromuscular assessments during initial exercise bout

Neuromuscular assessments were performed following each set (6 sets total) to determine the extent of fatigue-induced changes prior to the recovery intervention. Relative MVC torque was significantly lowered in both groups following sets 1-6, with the 6th set MVC torque values being 55.2 ± 26.1% and 49.5 ± 17.3% of the pre-exercise value for RT and CWI, respectively (Fig. 2a). There were no significant differences in the fatigue-induced reduction in the relative MVC torque between the two groups during the 6 sets (set 1: $p = 0.81$; set 2: $p = 0.86$; set 3: $p = 0.29$; set 4: $p = 0.46$; set 5: $p = 0.53$; set 6: $p = 0.48$). When assessing for changes in VA%, no differences were seen within or between the two groups throughout the 6 sets (set 1: $p = 0.36$; set 2: $p = 0.54$; set 3: $p = 0.32$; set 4: $p = 0.72$; set 5: $p = 0.75$; set 6: $p = 0.19$), suggesting that central fatigue was not apparent during SIE. Initial VA% was 98.6 ± 1.0% and 99.2 ± 1.0% for RT and CWI, respectively, and immediately after the 6th set, VA% was 96.9 ± 2.0% and 98.0 ± 2.1%, respectively (Fig. 2b). Regarding peripheral fatigue assessed via evoked contractions, in comparison to the pre-exercise value, 10Hz torque was significantly lowered following sets 1-6 for the CWI group and sets 2-6 for the RT group, whereas 50Hz torque decreased following sets 1-6 in both conditions. For 10Hz torque, pre-exercise torque values were 12.5 ± 3.7 Nm and 12.3 ± 3.2 Nm for RT and CWI, respectively, and after the 6th set being 3.6 ± 1.9 Nm and 2.8 ± 1.3 Nm with a significant difference between the two groups following set 3 only (set 1: $p = 0.31$; set 2: $p = 0.21$; set 3: $p = 0.047$; set

4: $p = 0.44$; set 5: $p = 0.09$; set 6: $p = 0.47$) (Fig. 3b). For 50Hz torque, beginning torques were 21.0 ± 7.2 Nm and 19.7 ± 6.4 Nm for RT and CWI, respectively, and after the 6th set being 13.3 ± 4.1 Nm and 10.4 ± 4.8 Nm with a significant difference between the two groups following set 4 only (set 1: $p = 0.47$; set 2: $p = 0.39$; set 3: $p = 0.34$; set 4: $p = 0.03$; set 5: $p = 0.14$; set 6: $p = 0.12$) (Fig. 3b). The 10:50Hz torque ratio was significantly different in the RT condition following sets 2-6 whereas for CWI, statistical differences were seen following sets 3-6. Beginning 10:50Hz torque values were 0.63 ± 0.14 and 0.66 ± 0.19 for RT and CWI, respectively, and following the final set was 0.29 ± 0.12 and 0.33 ± 0.20 (Fig. 3a). There were no significant differences between the two groups for the 10:50Hz torque ratio following any set (set 1: $p = 0.71$; set 2: $p = 0.49$; set 3: $p = 0.24$; set 4: $p = 0.63$; set 5: $p = 0.58$; set 6: $p = 0.95$).

3.3.3 Neuromuscular function following the recovery interventions

When comparing the relative recovery rates of MVC torque, both the RT and CWI conditions displayed significantly reduced MVC torque recovery rates throughout the first 3 hours of recovery which was then fully recovered 24 hours later (97.9 ± 7.4% and 103.7 ± 20.1% respectively) (Fig. 4a). No differences in MVC torque between the two groups were seen at any point throughout the 24-hour recovery period (0-h: $p = 0.40$; 0.5-h: $p = 0.20$; 1-h: $p = 0.12$; 3-h: $p = 0.15$; 24-h: $p = 0.68$). Similarly to the initial bout of SIE, no significant changes occurred with VA% throughout the recovery period duration (0-h: $p = 0.59$; 0.5-h: $p = 0.63$; 1-h: $p = 0.81$; 3-h: $p = 0.74$; 24-h: $p = 0.46$) (Fig. 4b). Looking at the recovery of 10Hz torque, both groups saw significant reductions following exercise, with both groups displaying depressed 10Hz torque for the initial 3 hours of recovery with no differences between the two groups (0-h: $p = 0.16$; 0.5-h: $p = 0.48$; 1-h: $p = 0.45$; 3-h: $p = 0.92$) (Fig. 5b). It was not until 24 hours later that 10Hz torque had recovered for both the RT and CWI conditions (11.3 ± 4.3 Nm and 10.2 ± 2.0 Nm respectively; p

= 0.95) (Fig. 5b). When comparing the recovery of 50Hz torque, the RT condition was shown to recover immediately following the recovery intervention (16.5 ± 6.2 Nm), whereas compared to the CWI condition, it remained impaired for 3 hours following the intervention (14.9 ± 6.0 Nm) and did not display recovery until 24 hours later (18.8 ± 6.7 Nm) (Fig. 5b). However, no significant differences were seen between the two groups throughout the recovery period (0-h: $p = 0.49$; 0.5-h: $p = 0.26$; 1-h: $p = 0.63$; 3-h: $p = 0.30$; 24-h: $p = 0.29$). Finally, when comparing the recovery of the 10:50Hz torque ratio, the RT condition remained impaired for up 3 hours (0.53 ± 0.15) and did not fully recover until 24 hours later (0.58 ± 0.16), whereas the CWI condition was able to recover within 30 minutes (0.53 ± 0.31) (Fig. 5a). No significant differences were seen between the 10:50Hz torque ratio for the RT and CWI conditions, however, there was a trend toward a greater 10:50Hz torque ratio at the post-intervention times of 30 minutes, 1 hour, and 3 hours of the recovery period (0-h: $p = 0.11$; 0.5-h: $p = 0.058$; 1-h: $p = 0.067$; 3-h: $p = 0.087$; 24-h: $p = 0.97$) (Fig. 5a).

3.3.4 Intramuscular temperature changes during exercise and recovery

When looking at the initial bout of SIE, no differences in intramuscular temperature were seen between the two groups ($p = 0.33$). In the RT group, exercise increased intramuscular temperature by 2.0 ± 0.4˚C to 35.6 ± 0.9˚C post-exercise (Fig. 6b). In the CWI group, intramuscular temperature was increased by 2.2 ± 0.4˚C during exercise to 36.0 ± 0.7˚C post-exercise (Fig. 6b). During the recovery interventions, the RT condition decreased intramuscular temperature 0.2 ± 0.3˚C compared to the CWI, which displayed a significant decrease in temperature by 3.7 ± 1.7˚C ($p < 0.0001$) (Fig. 6b). During the initial 3 hours following the recovery intervention, significant differences in intramuscular temperature were seen for the first 102 minutes of recovery ($p < 0.05$), with nonsignificant but noticeable differences thereafter for the

remaining time (Fig. 6a). Finally, when comparing the temperature changes spanning the 3-hour post-recovery intervention period, a significant reduction in intramuscular temperature was seen for the RT condition compared to CWI (2.8 ± 0.8˚C vs. 0.2 ± 2.2 ˚C; $p = 0.0009$) (Fig. 6b).

3.3.5 Performance between bout 1 and bout 2 of SIE separated 24-hours apart

When comparing performance between the groups, the ability to maintain peak isokinetic speed at 160º/sec was used to determine fatigue resistance. Although mean peak velocity could not be maintained with increasing sets across all bouts of SIE (Fig. 7), there was no difference in mean peak velocity (Table 2), or total contractions performed per set (Table 3) during the initial bout or next day bout of SIE between the two conditions. Therefore, no difference in fatigability was seen at any point during bout 1 or 2 of SIE.

3.4 Discussion

This study set out to explore the effects of cold-water immersion following SIE on muscle recovery across a 24-hour period. Furthermore, performance was assessed 24 hours apart to understand the prolonged effects that CWI might have on subsequent exercise performance. The major findings indicate that CWI was effective in accelerating the recovery of skeletal muscle at the expense of maximal tetanic force (Fig. 5). By impairing 50Hz maximal tetanic torque, and maintaining 10Hz submaximal tetanic torque, the 10:50Hz torque ratio subsequently increased suggesting favorable recovery. However, in circumstances such as this, impairing maximal muscle force generation is less than favorable for optimal recovery.

We originally hypothesized that the accelerated recovery of skeletal muscle following CWI would occur due to acute recovery of the 10Hz submaximal tetanic torque. Mentioned earlier, previous studies have shown that following HIE, PLFFD is present and has been shown to remain

impaired for considerably longer than high frequency forces (Fernandez-del-Olmo et al., 2013; Place et al., 2015) due to the presence of exercise-induced oxidative stress. It was thought that CWI following fatiguing exercise would acutely recover low frequency force by reducing post-exercise ROS generation and intramuscular temperature (White & Wells, 2013) and slowing and delaying the recovery of SR Ca^{2+} release and reuptake (Halder & Gao, 2014), thus prolonging relaxation time (Power et al., 2016) between evoked contractions leading to greater force fusion. However, acute 10Hz submaximal tetanic force recovery was not seen following CWI (Fig. 5b). Interestingly, the effect of CWI seemed to have delayed the recovery of high-frequency force instead, which was shown to remain impaired for up to 3 hours compared to RT, which recovered immediately (Fig. 5b). Of the many suggested benefits for CWI, one of the potential detrimental effects of cooling skeletal muscle is its effects on the rate of excitation-contraction coupling (White & Wells, 2013). Specifically, CWI has been shown to prolong action potential propagation thus resulting in delayed and slowed contraction velocity and impaired rate of force production and tension development (Cè et al., 2012; Drinkwater & Behm, 2007; Halder & Gao, 2014; Howard et al., 1994; White & Wells, 2013). Furthermore, it has been shown previously that maximal effort contractions are more affected at reduced muscle temperatures compared to submaximal effort contractions (Ferretti, 1992). This reduced intramuscular temperature impairment to maximal effort contractions has to do with the rate of force production, which is correlated to the rate of ATP hydrolysis, and it is suggested that during submaximal contractions, ATP can be resynthesized at a slower rate compared to maximal contractions (Ferretti, 1992). Thus, it can be speculated that CWI may have impaired the rate of peak torque development during high-frequency contractions, which is consistent with previous studies showing impaired power output (Choo et al., 2022;

Crampton et al., 2014) and increased sprint time (Chaillou et al., 2022; Parouty et al., 2010) during the acute phase of recovery following CWI.

Unlike the evoked maximal tetanic contractions, MVC torque was impaired following both RT and CWI recovery interventions for up to 3 hours (Fig. 4a). However, inconsistencies exist within the literature regarding MVC torque recovery following cooling conditions. A study performed in the forearm muscles showed that MVC force was reduced in cold temperatures compared to neutral or hot temperatures (Mallette et al., 2021) whereas another study in the elbow flexors showed no change in MVC force under cooled, neutral, or hot temperatures (Cè et al., 2012). It has been shown that dynamic power contractions are more sensitive to temperature changes than isometric contractions (Howard et al., 1994), suggesting that CWI at 10ºC for 10 minutes following skeletal muscle fatiguing exercise might not have been enough of a cooling stimulus to alter MVC torque in the ankle dorsiflexor muscles, as it has been shown previously that the extent of skeletal muscle cooling can further impair tetanic force (Coupland & Ranatunga, 2003).

Regarding VA%, our SIE model performed in the ankle dorsiflexor muscles was enough to induce muscle fatigue, however it was not enough to elicit signs of central fatigue indicated by the preservation of VA%. Similarly, the study by Place et al. (2015) involved repeated Wingate exercise performed on a cycle ergometer, thus involving a greater muscle mass and rise in intramuscular and core body temperature, also showed no presence of central fatigue. It has been shown previously that during brief isometric contractions under cooled intramuscular temperatures, a brief isometric contraction (3-sec) does not show a deficit in VA% (Lloyd et al., 2015). However, in that same study, prolonged sustained isometric contractions (120-sec) and associated VA% was reduced when under cooled intramuscular temperatures. Therefore, during

our neuromuscular assessments, VA% was assessed during a brief 5 second MVC suggesting that even if VA% would have been impaired following SIE, a brief 5 second MVC might not have been long enough to detect differences in VA% between our CWI and RT conditions compared to more prolonged and sustained MVC's. Nonetheless, our SIE model showed no signs of central fatigue and CWI had no effect on VA% (Fig. 4b).

When considering the effects of post-exercise CWI on subsequent SIE performed 24 hours later, no differences were seen in either the pre-exercise recovery of neuromuscular function nor in isokinetic velocity and total contractions per set assessed during either bout of exercise (Fig. 7). It is thought that the effects of CWI would have worn off well before the second bout of exercise due to the reperfusion of blood flow and restoration of intramuscular temperature, suggesting that CWI had no effect on next day SIE performance and fatigue resistance. In contrast, previous studies have shown improved next day sprint repeatability (Leeder et al., 2019; Tabben et al., 2018) yet impaired power output (White et al., 2014). Inconsistencies in next day sprint exercise might be due to the selected sprint test used to assess sprint repeatability. Specifically, Leeder et al. (2019) used the Loughborough Intermittent Shuttle Test to determine sprint repeatability, which is a varied exercise intensity test, which might not represent all-out effort typically seen with SIE. Furthermore, the study by Tabben et al. (2018) used a 10 metre sprint to determine sprint repeatability performance which is considerably shorter in duration to our SIE model which involved 3 minutes of total work (6 sets x 30-sec intervals) per SIE bout. Therefore, future studies are still needed to determine the prolonged effects that CWI might have on subsequent HIE performance.

Some limitations exist with our study which includes the absence of EMG data. However, it was shown by Place et al. (2015) that VA% was relatively unaffected with HIIE indicative of the

unchanged M waves. Furthermore, using a modified repeated Wingate exercise protocol in the ankle dorsiflexors, VA% remained unchanged throughout the protocol's entirety. Thus, it is fair to speculate that fatigue experienced following our exercise protocol all resided intrinsic to the muscle fibers, and any cooling related recovery or impairment would have also resulted intrinsically. Another limitation of our study was the exclusion of contractile power assessments throughout the 24-hour recovery period, which are functionally relevant to locomotion and provide a measure of the ability to contract rapidly while developing force during shortening and lengthening. Power output assessments have been shown to be impaired following CWI both acutely (Choo et al., 2022; Crampton et al., 2014) and at more prolonged stages of recovery (White et al., 2014). Therefore, this study set out to explore and differentiate central versus peripheral fatigue recovery to determine the effects that CWI has on muscle-dependent recovery following SIE, which has not been shown previously. Another potential limitation of this study is the use of ankle dorsiflexors instead of a larger muscle group, such as the knee extensors. It was thought that by choosing a smaller muscle group with a smaller muscle mass, our selected CWI protocol would be able to reduce intramuscular temperature to a greater extent thus providing more insight into post-exercise cooling related effects on skeletal muscle recovery. Furthermore, the fibre type distribution of muscle has been shown to be affected with repeated exposure to CWI. A study by Fyfe et al. (2019) showed that 7 weeks of regular exercise and CWI significantly impaired type II muscle hypertrophy compared to type I with no impedance to maximal strength development in both. However, type I muscle fibres are preferentially used during endurance type exercise and elicit greater capillarity adaptations with training (Prior et al., 2004) and repeated exposure to CWI has been shown to impair vascular remodelling (White & Wells, 2013), thus suggesting that CWI might affect muscles to a greater extent than others based on fibre type distribution. Lastly, another

limitation with CWI studies is the lack of participant blinding. A study by Broatch et al. (2014) showed that psychological factors can have an influence on participant MVC force. Specifically, when participants performed either thermoneutral water immersion, CWI, or a thermoneutral water immersion placebo condition with a dissolvable skin cleanser that participants were led to believe was beneficial to recovery, it was both the CWI and placebo condition that displayed the greatest MVC torque recovery following HIE. Therefore, it seems that a placebo effect might exist for participants who believe a certain way regarding CWI as an effective recovery modality.

In conclusion, CWI following SIE resulted in acute skeletal muscle recovery at the expense of maximal tetanic force. Impaired 50Hz torque following CWI persisted for up to 3 hours, whereas RT recovered immediately. It is thought that impaired 50Hz torque has to do with impaired rates to peak torque development due to significantly lowered intramuscular temperature following CWI compared to RT. Furthermore, impaired maximal tetanic force following CWI was fully recovered 24 hours later, suggesting that any potential prolonged effects of CWI on neuromuscular fatigue recovery dissipate before then due to intramuscular temperature returning to baseline within hours following the recovery intervention. Lastly, CWI had no effect on next day SIE performance as fatigue resistance was similar between the two conditions regarding peak velocity performance and total contractions per set.

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Figure 1.

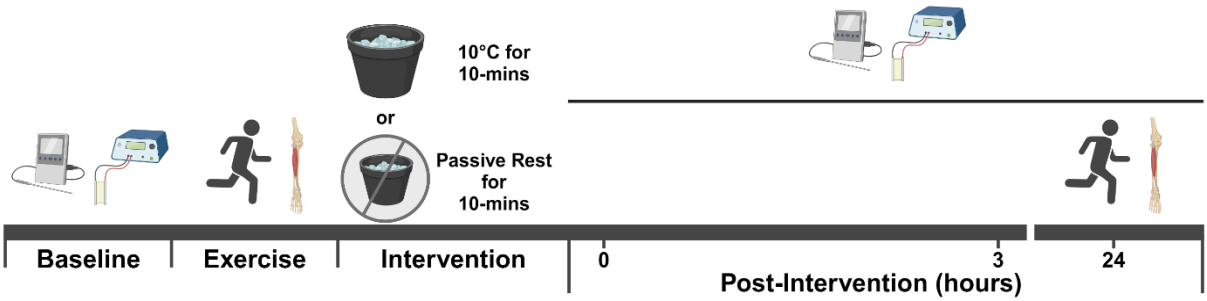


Figure 1. Experimental protocol. Study schematic representing an outline of the experimental procedures during one condition.

Figure 2.

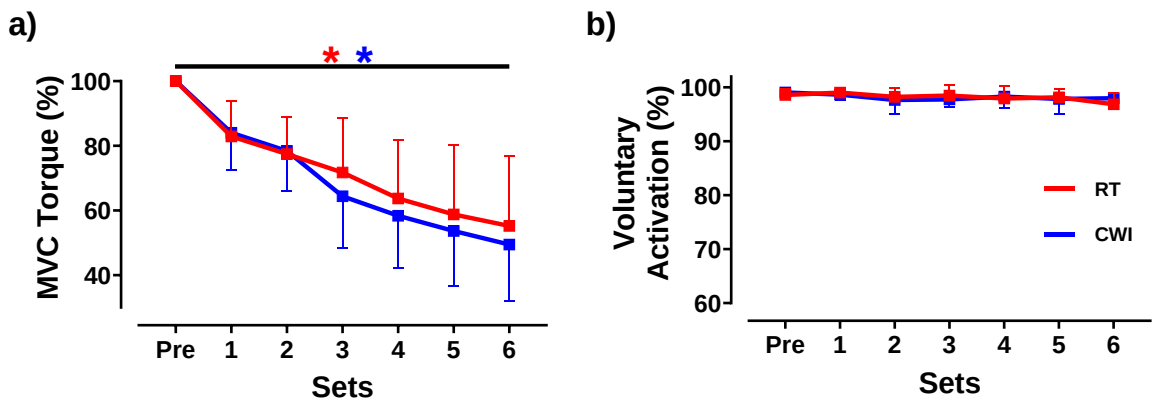


Figure 2. a) MVC torque (%) assessed at pre and immediately following each exercise set. **b)** Voluntary activation (%) assessed at pre and immediately following each exercise set. The red asterisk signifies significant reductions in torque in the RT group from pre and the blue asterisk denotes significant reductions in torque compared to pre in the CWI group.

Figure 3.

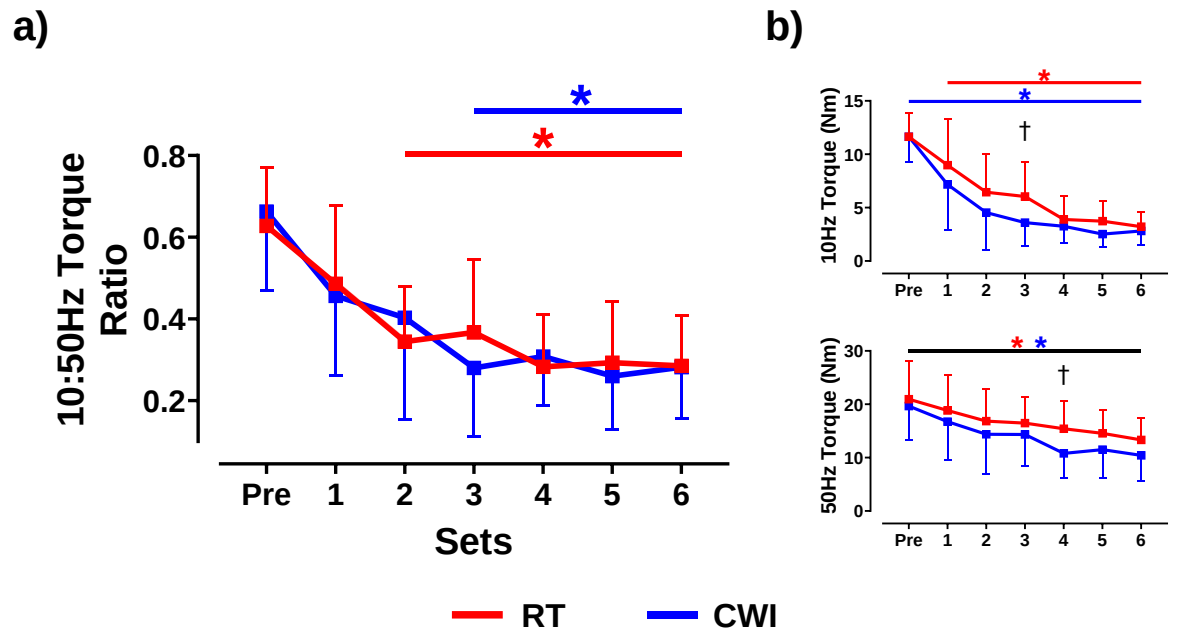


Figure 3. a) 10:50Hz torque ratio assessed at pre and immediately following each exercise set. **b)** 10Hz torque (Nm) (Upper) and 50Hz torque (Nm) (Lower) assessed at pre and immediately following each exercise set. The red asterisk signifies significant reductions in torque in the RT group from pre and the blue asterisk denotes significant reductions in torque compared to pre in the CWI group.

Figure 4.

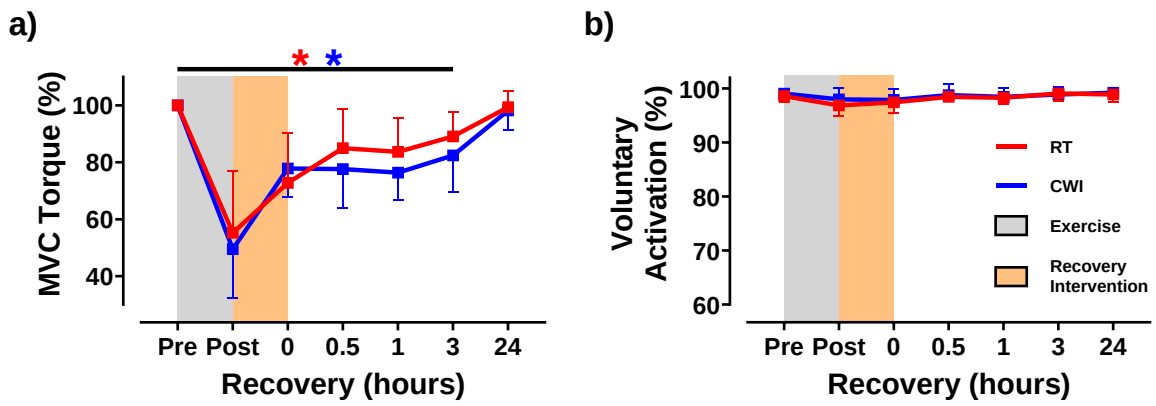


Figure 4. a) MVC torque (%) assessed at pre and immediately following SIE (grey bar), the recovery intervention (tan bar), and throughout a 24-h recovery period beginning at 0-h. **b)** Voluntary activation (%) assessed at pre and immediately following SIE (grey bar), the recovery intervention (tan bar), and throughout a 24-h recovery period beginning at 0-h. The red asterisk signifies significant reductions in torque in the RT group from pre and the blue asterisk denotes significant reductions in torque compared to pre in the CWI group.

Figure 5.

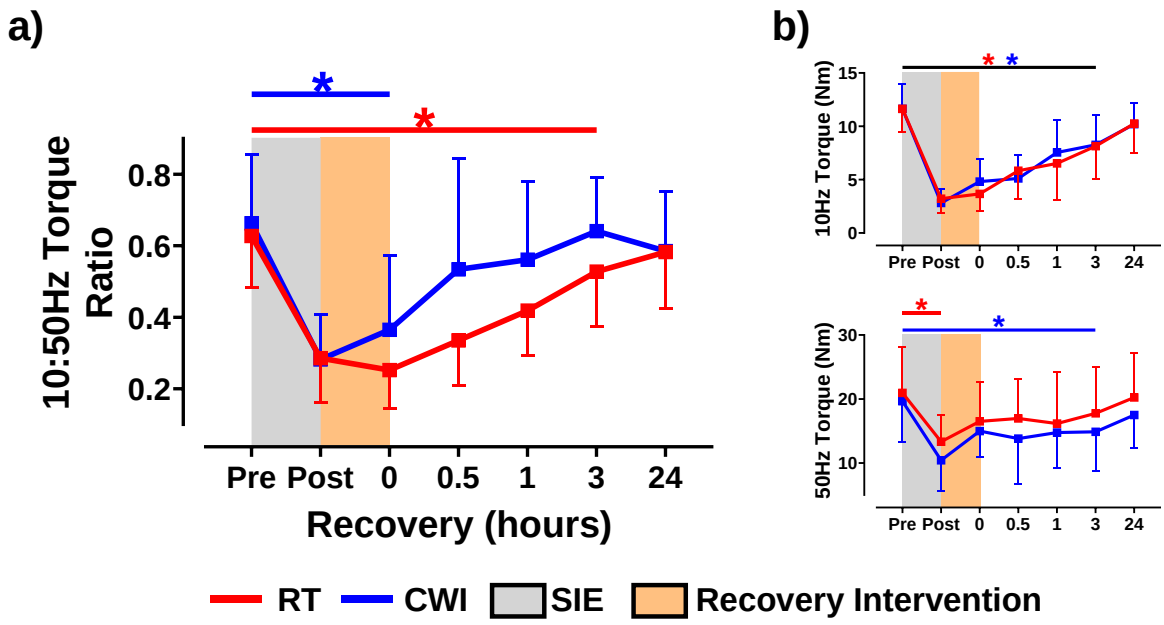


Figure 5. a) 10:50Hz torque ratio assessed at pre and immediately following SIE (grey bar), the recovery intervention (tan bar), and throughout a 24-h recovery period beginning at 0-h. **b)** 10Hz torque (Nm) (Upper) and 50Hz torque (Nm) (Lower) assessed at pre and immediately following SIE (grey bar), the recovery intervention (tan bar), and throughout a 24-h recovery period beginning at 0-h. The red asterisk signifies significant reductions in torque in the RT group from pre and the blue asterisk denotes significant reductions in torque compared to pre in the CWI group.

Figure 6.

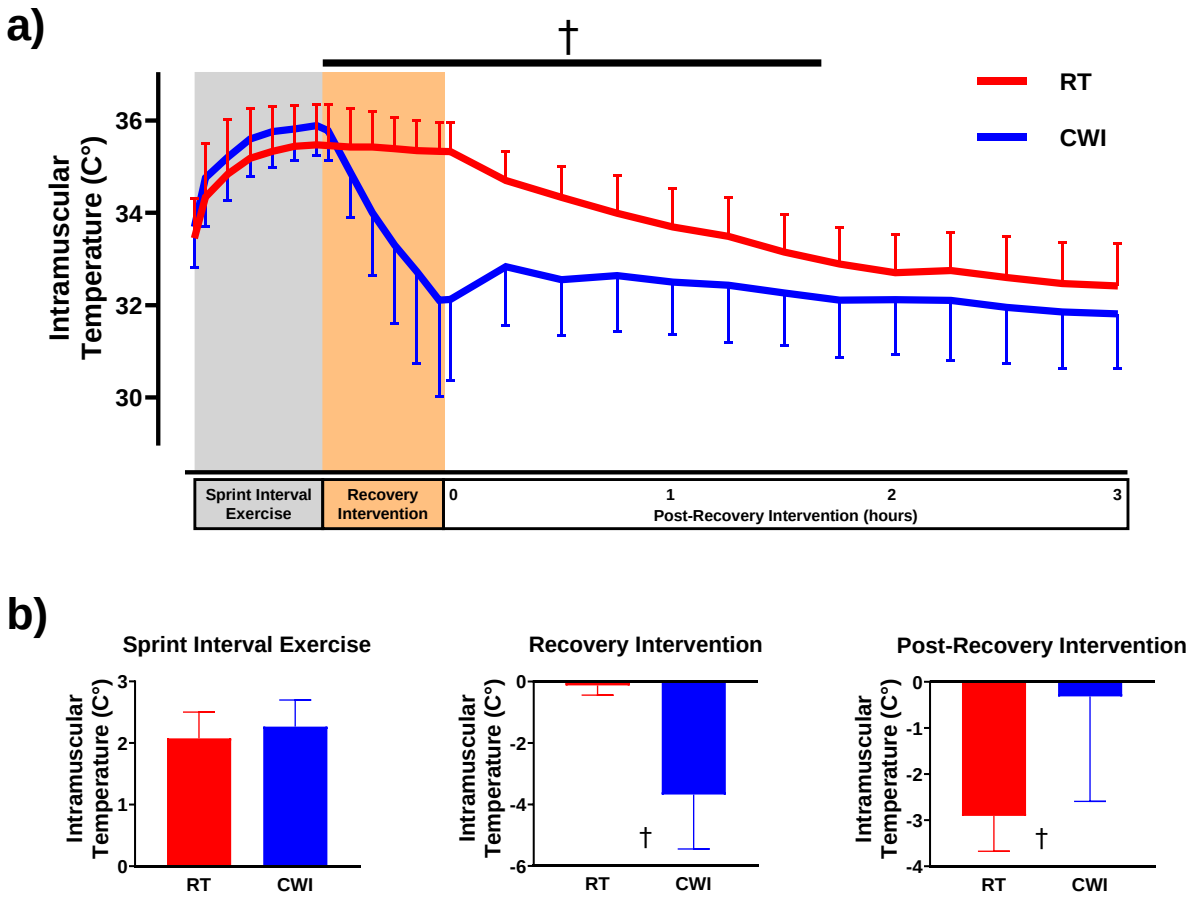


Figure 6. a) Intramuscular temperature changes tracked during SIE, the recovery interventions, and 3 hours of the post-recovery interventions. **b)** Intramuscular temperature during SIE comparing pre to peak temperatures (Left). Intramuscular temperature during both recovery interventions comparing pre to post temperatures (Middle). Intramuscular temperature during the post-recovery intervention comparing the difference from 0-h to 3-h (Right). The cross denotes a significant difference in intramuscular temperature.

Figure 7.

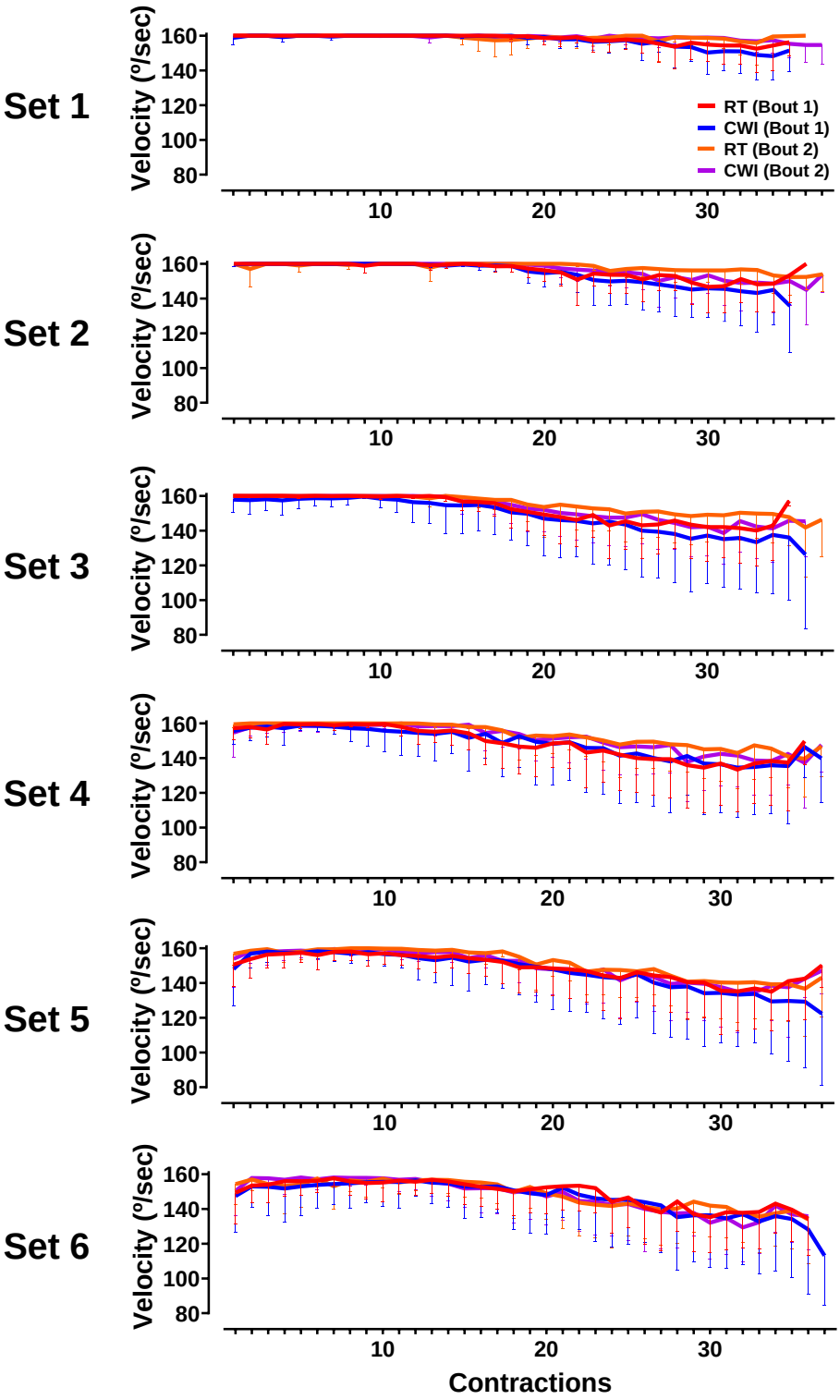

Set 1
Set 2
Set 3
Set 4
Set 5
Set 6
Velocity (°/sec)
160
140
120
100
80
10
20
30
Contractions
RT (Bout 1)
CWI (Bout 1)
RT (Bout 2)
CWI (Bout 2)

Figure 7. Performance. Fatigue resistance assessed as the ability to maintain peak isokinetic velocity set at 160º/sec. Mean contractions achieved during each bout and per set were used to determine mean peak velocity. No difference was seen in fatigue resistance between either exercise bout or group.

Table 1. Baseline comparison.

	RT	CWI	t-test *p*-value
MVC Torque (Nm)	**34.8 ± 8.4**	**34.4 ± 10.0**	**0.80**
VA (%)	**98.5 ± 1.0**	**99.2 ± 1.0**	**0.10**
10Hz Torque (Nm)	**12.5 ± 3.7**	**12.3 ± 3.2**	**0.66**
50Hz Torque (Nm)	**21.0 ± 7.2**	**19.7 ± 6.4**	**0.23**
10:50Hz Torque	**0.63 ± 0.14**	**0.66 ± 0.19**	**0.38**
Intramuscular Temperature (ºC)	**33.5 ± 0.9**	**33.7 ± 0.9**	**0.41**

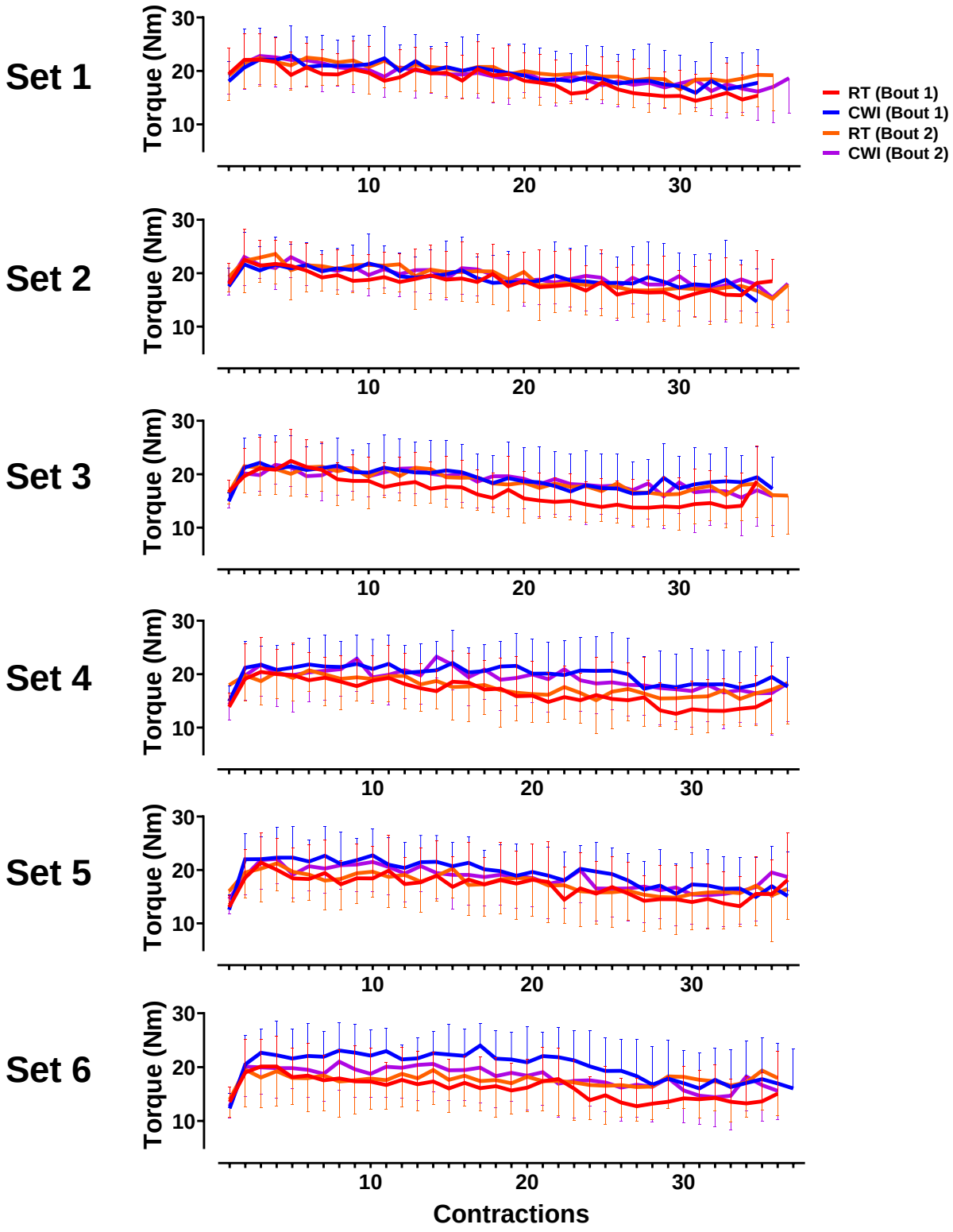
Table 2. Comparison of total peak velocity achieved.

		RT	CWI	t-test *p*-value
Bout 1	Set 1	158.1 ± 3.7	156.7 ± 3.9	0.24
	Set 2	155.9 ± 5.0	153.5 ± 7.8	0.39
	Set 3	152.0 ± 7.7	148.0 ± 16.9	0.48
	Set 4	148.2 ± 10.6	147.8 ± 16.8	0.94
	Set 5	148.5 ± 11.7	146.6 ± 17.6	0.73
	Set 6	148.7 ± 10.7	146.2 ± 19.9	0.71
Bout 2	Set 1	159.0 ± 3.2	158.9 ± 2.4	0.84
	Set 2	158.2 ± 2.4	156.3 ± 4.8	0.17
	Set 3	154.9 ± 7.8	152.2 ± 8.0	0.37
	Set 4	153.3 ± 7.9	150.8 ± 8.6	0.37
	Set 5	151.5 ± 8.6	149.1 ± 10.2	0.39
	Set 6	148.6 ± 10.7	147.1 ± 10.9	0.66

Table 3. Comparison of total contractions performed.

		RT	CWI	t-test *p*-value
Bout 1	Set 1	35.7 ± 3.2	35.6 ± 2.4	0.97
	Set 2	36.0 ± 3.5	35.8 ± 2.3	0.85
	Set 3	35.8 ± 3.5	36.1 ± 2.9	0.76
	Set 4	35.7 ± 3.9	36.1 ± 2.5	0.73
	Set 5	36.0 ± 3.3	36.0 ± 1.9	>0.99
	Set 6	36.0 ± 3.6	37.0 ± 2.7	0.33
Bout 2	Set 1	36.7 ± 3.7	37.3 ± 2.7	0.55
	Set 2	37.0 ± 3.6	37.1 ± 2.7	0.94
	Set 3	37.2 ± 3.8	36.7 ± 2.6	0.66
	Set 4	36.9 ± 3.7	36.5 ± 3.0	0.71
	Set 5	36.4 ± 3.2	36.5 ± 3.1	0.93
	Set 6	36.1 ± 3.0	36.3 ± 2.7	0.86

Supplemental Figure 1. Peak torque per contraction across sets.



Supplementary Figure 2. Neuromuscular assessments pre- versus post-SIE for bout 1 and 2.