THE EFFECTS OF WHOLE-BODY HOT WATER IMMERSION AFTER EXERCISE INDUCED MUSCLE DAMAGE ON EXERCISE PERFORMANCE AND INFLAMMATORY MARKERS

Rogelio A. Realzola
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THE EFFECTS OF WHOLE-BODY HOT WATER IMMERSION AFTER EXERCISE INDUCED MUSCLE DAMAGE ON EXERCISE PERFORMANCE AND INFLAMMATORY MARKERS

by

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DISSEMINATION
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ABSTRACT

This study examined the effects of hot water immersion (HWI) on exercise performance and inflammatory markers after a resistance exercise-induced muscle damage protocol. Strength trained males \(n = 5\) and females \(n = 5\) completed two experimental trials separated by at least 2 weeks in a randomized crossover design; one trial involved HWI, the other temperate water immersion (TWI). For each trial, subjects performed a muscle damaging protocol followed by HWI or TWI for up to 50 minutes. The protocol consisted of 10 sets of 8 repetitions at 70% of 1-repetition maximum with 4s eccentric, 1s concentric lifting tempo, followed by 4 sets of 20 plyometric lunges. Vertical jump, isometric and isokinetic maximal voluntary contractions, and blood markers of muscle
damage and inflammation were measured prior to, and at 24h-, 48h-, and 72h-post exercise. No decreases in jump height, and isometric maximal voluntary contraction (MVC) were observed for HWI and TWI. For isokinetic MVC at 180°/s, HWI showed no decreases at any time point, while TWI decreased at every time point compared to baseline. HWI and TWI isokinetic MVC at 60°/s decreased at all time points. Soreness was increased at every time-point after HWI and TWI when compared to baseline. For blood markers, compared to baseline, creatine kinase (CK) increased at 24h-post-exercise, but was not different from baseline at 48h- and 72h-post exercise after HWI. In TWI, CK was increased at 24h-, and 72h-post exercise when compared to baseline. Myoglobin was only increased after HWI at 24h-post exercise. For inflammatory markers, C-reactive protein was increased at 24h-post exercise for HWI, but not TWI. Tumor necrosis factor-α was increased 48h- and 72h-post exercise when compared to baseline for HWI but was not increased at any time point for TWI. On the other hand, Interleukin-6 was increased 24h- and 48h-post exercise for TWI but was not increased at any time point for HWI. No changes in Interleukin-10 were observed. These findings indicate that HWI is marginally more effective than TWI to hasten exercise performance and reduce creatine kinase levels. Conversely, we speculate that inflammation lasted longer in the HWI.
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Chapter I: Introduction

Post-exercise recovery is a multifaceted process that is affected by time, determined by training load, training history, subject to individual responses, and is heavily influenced by the demand of the sport or physical activity (Kellmann et al., 2018). Growing demands of sports, athletic performance and the rising importance of accelerating recovery have prompted discovery and use of various strategies to facilitate or accelerate recovery (Kellmann et al., 2018). These recovery strategies are important, as a functional, non-overreaching amount of fatigue is important for training adaptations but can also be deleterious to performance. Recovery strategies, then, are important for ameliorating exercise-induced muscle damage.

Exercise-induced muscle damage (EIMD) is typically experienced following prolonged and/or high-intensity exercise, collision sports, eccentric muscle loading, and unaccustomed exercises (Sabapathy et al., 2021; Stožer et al., 2020a; Tidball & Villalta, 2010). EIMD is related to disruptions of subcellular skeletal muscle structures and can be characterized by loss of muscular function measured by maximal voluntary contraction (MVC), losses in power production, delayed onset muscle soreness (DOMS), swelling of the affected area, and/or reduced range of motion (Hyldahl & Hubal, 2014). Further, increases in specific muscle proteins detected in the blood (creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin(Mb)) are considered indirect markers of muscle damage (Hyldahl & Hubal, 2014). As increased markers of EIMD can translate to decrements in performance, there exists various recovery strategies that can aid in facilitating recovery.
Recovery strategies – practices that are intended to increase the rate of recovery – can be separated into two categories: type and time. Specifically, the type can be classified as either active or passive, while time is classified as pre- (priming or prophylactic use) or post-training session (recovery). Briefly, passive recovery entails application of external methods and/or implementing a state of rest characterized by inactivity, while active recovery involves physical activities (Kellmann et al., 2018). Importantly, each recovery strategy has its own specific way in mediating or accelerating recovery. Passive recovery modalities, such as hot or cold water immersion, saunas, and/or massage therapy may involve mitigation of inflammatory responses and/or altering nervous system sensitivity (Nosaka et al., 2007; Owens et al., 2019; Thorpe, 2021). On the other hand, active recovery modalities, such as cooldown jogging or cross-training are aimed at increasing blood flow to “shuttle” away metabolic byproducts such as reactive oxygen species (ROS), nitric oxide species (NOS), and hydrogen ion (H+) accumulation, which may play a role in inducing or prolonging fatigue following exercise (Hyldahl & Peake, 2020; Kellmann et al., 2018; Thorpe, 2021).

Regarding timing, examples of pre-exercise heating or vibration have been shown to positively affect EIMD through facilitation of blood flow and increased trafficking of neutrophils and macrophages (Davis et al., 2020, Nosaka et al., 2007). An increase in neutrophils and macrophages may be responsible for the hypersensitization of nociceptors within the muscle. Thus, attenuation of trafficking may be beneficial for performance decrements and soreness Saga et al., 2008; Touchberry et al., 2012). Still, other evidence suggests that there may be no change or even larger decrements in power production, force loss, and soreness with the use of heat or vibration (Dupuy et al., 2018;
Post-exercise recovery strategies are mainly focused on attempting to facilitate performance recovery by restoring impairments in force loss and contractility due to decrements in muscle glycogen resynthesis, enhancing adenosine triphosphate (ATP) turnover and the consequential increase in intracellular signaling pathways, as well as decreasing soreness via flushing out factors that may cause an increase in hypersensitization (Kim et al., 2019; Stožer et al., 2020b). In a more practical sense, recovery might entail return to baseline performance for isometric and isokinetic markers, jump height, 1-repetition maximum, or decreased soreness. While newer technology like compressive garments, hyperbaric therapy, neuromuscular electrical stimulation, and percussive therapy are less studied, the most common post-exercise treatment for EIMD is, arguably, passive thermal recovery (Cullen et al., 2021; Hyldahl & Peake, 2020; Kellmann et al., 2018; Thorpe, 2021). Passive thermal recovery strategies, such as cold-water immersion (CWI), saunas, and localized thermal application (e.g. heating pads, diathermy, and ice packs) have been studied extensively, although results on EIMD are mixed due to the lack of consensus in their application (Cullen et al., 2021; Kellmann et al., 2018; Laudner et al., 2013; McGorm et al., 2018; Ortiz et al., 2019; Thorpe, 2021). Indeed, the lack of agreement of when (pre- vs post-exercise), at what temperatures (hot vs cold), and for how long makes it difficult to effectively apply an optimal recovery strategy. Importantly, the use of supplements or nutritional strategies can be viable options as well. Although, similar to the aforementioned recovery strategies, a lack of agreement on dosing, time, and effectiveness exists. Several nutritional strategies will be mentioned in later chapters.
Thus, while an array of different strategies can be used to improve recovery acutely, there are potential drawbacks to modalities that may limit adaptations with prolonged use.

Cryotherapy is a therapeutic process involving local or whole-body heat extraction through cold water immersion (CWI), whole-body cryotherapy (WBC), and localized cooling/ice application (Allan et al., 2022). While early research has been unclear about the mechanisms behind how recovery occurs, it is clear that cryotherapy decreases DOMS, but may play a small, if any, role in accelerating recovery of muscle contractile performance (Hohenauer et al., 2015). Changes in recovery processes after EIMD are thought to be due to reductions in body temperatures and the associated perturbations in central nervous system (CNS) fatigue, reducing cardiovascular strain, stimulating vasoconstriction, altering subsequent leukocyte trafficking and activity, and decreasing muscle soreness and swelling (Ihsan et al., 2016; Kwiecien & McHugh, 2021). These physiological changes are proposed to be the reasoning behind reductions in soreness for up to 96 hours post-exercise, and a reduction in CK, LDH, and Mb (Dupuy et al., 2018; Hohenauer et al., 2015). Recently, molecular and physiological changes due to CWI have been investigated, mainly due to the negative effects on strength and muscle mass that occurs with prolonged (4-12 weeks) use (Fyfe et al., 2019; Roberts et al., 2015). Roberts et al. (2015) showed that CWI after resistance training sessions reduced regulatory muscle hypertrophy kinases (p70S6) activation, reduced satellite cell expansion and substantially attenuated long-term gains in muscle mass and strength. Further, it is possible that reductions in muscle temperature may negatively influence the expressions of genes or activity of transcription factors that regulate muscle growth (Roberts et al., 2015). This may be due to cold shock proteins inhibiting satellite cells
from differentiating by decreasing myogenin expression (Roberts et al., 2015).

Importantly, although occurring in Type I muscle fibers, this may be more prominent in Type II muscle fibers, as CWI following 7 weeks of resistance training coincided with post-exercise decreases in mammalian target of rapamycin complex 1 (mTORC1) signaling and increased levels of transcription factors related to muscle protein degradation (e.g., forkhead box protein O1 (Fox-O1)) (Fyfe et al., 2019). Other key proteins that regulate muscle protein synthesis (i.e., 4E-BP1 and ribosomal protein s6 (rps6)) were not affected in cold though, suggesting that decrements in hypertrophy, despite a resistance training program, may occur for reasons other than CWI (Fyfe et al., 2019). Still, attenuation of muscle hypertrophy due to CWI is potentially independent of changes in factors that regulate myogenesis, proteolysis, and extracellular matrix remodeling in muscle after exercise (Peake et al., 2020). Thus, while CWI may be beneficial for mitigating soreness, and presumably increasing training adherence, potential interference of hypertrophic processes should lead to caution for the use of CWI as a recovery strategy, especially during a resistance training protocol (Fyfe et al., 2019; Hyldahl & Peake, 2020; Roberts et al., 2015).

Given the potential negative long-term effects of cryotherapy post-exercise on strength and hypertrophy, heat therapy has been considered as an alternative thermal therapy. It is possible that heat has the potential to have acute positive effects on EIMD after a damaging protocol, as well as long-term benefits for strength and hypertrophy. Heating modalities can include whole-body hot water immersion, and more targeted tissue strategies such as local water immersion, ultrasound, and diathermy (EVANS et al., 2002; K. Nosaka et al., 2007; Symons et al., 2004; Vardiman et al., 2015). Pre-exercise
heating can elevate muscle temperatures to decrease strain, attenuating swelling, and increasing muscle and connective tissue extensibility (EVANS et al., 2002; Kazunori Nosaka et al., 2004). It is important to note that little to no differences have been found using pre-heating strategies following an EIMD protocol on recovery in MVC (Castellani et al., 2016; Nosaka et al., 2004, 2007; Symons et al., 2004), blood markers of EIMD (Castellani et al., 2016; Nosaka et al., 2007; Skurvydas et al., 2008; Vardiman et al., 2015a), strength loss (Nosaka et al., 2007; Symons et al., 2004), soreness (Nosaka et al., 2004), and markers of inflammation (Castellani et al., 2016). Thus, post-exercise heating is the next logical step in finding potential benefits in decreasing EIMD. Relatively few resistance exercise studies have tested post-exercise heating interventions and their effects on EIMD (Castellani et al., 2016; Kim et al., 2019; Petrofsky et al., 2017; Vaile et al., 2008b; Vardiman et al., 2015b). Still, the proposed mechanism for the benefit of heat post-exercise is through removal and enhanced transportation of metabolic byproducts and modulation of cellular healing, hemodynamics, and substrate resynthesis (McGorm et al., 2018). These changes may be due to an increase in blood flow, heart rate, cardiac output, and a decrease in peripheral resistance that is associated with heat (Wilcock et al., 2006). Importantly, post-exercise heating has not been found to attenuate the inflammatory process or mTOR signaling that accompanies muscular damage, meaning it may not cause decrements in acute and chronic adaptations to resistance exercise (Kim, Monroe, et al., 2020b). Thus, heat may be a better alternative to cryotherapy post-resistance exercise. Given the lack of data on hot water immersion (HWI) post-resistance exercise, the primary aim of this study is to investigate the effects of HWI on performance markers, indirect markers of muscle damage, and the inflammatory process
Problem Statement

It was previously reported CWI can accelerate recovery acutely, but long-term adaptations were blunted. As such, HWI is emerging as a potential modality to accelerate acute recovery while potentially benefiting long-term strength and/or hypertrophy gains. The benefits of HWI on recovery after resistance exercise are unclear. Therefore, it is currently unknown if HWI after a session of resistance exercise can ameliorate EIMD. Further, inflammatory markers that may give insight to the first and secondary phases of muscular damage have not been reported. As such, the current study will seek to determine the effect post-resistance exercise HWI has on performance markers, markers of EIMD, and the inflammatory response.

Purpose of the study

The purpose of this study is to evaluate the effects of whole-body HWI (42° C) and whole-body temperate water immersion (TWI -32° C) on markers of exercise performance (vertical jump and MVC), exercise-induced muscle damage (CK and Mb) and inflammation (C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-10 (IL-10), tumor necrosis factor alpha (TNFα)) after an eccentric-based resistance exercise protocol.

Hypotheses

Hypothesis 1: HWI will accelerate recovery of performance markers (MVC, and vertical jump) sooner than TWI at 24h-, 48h-, and 72h-post muscle-damaging exercise.

Rationale: Previous research has demonstrated that HWI improves MVC after resistance exercise (Vaile et al., 2008b).
Hypothesis 2: HWI will decrease markers of muscle damage sooner than TWI at 24h-, 48h, and 72h-post muscle-damaging exercise.

_Rationale:_ Previous research has demonstrated CK and Mb are decreased when compared to control utilizing a heating treatment after muscle-damaging resistance exercise (Vaile et al., 2008a; Viitasalo et al., 1995).

Hypothesis 3: HWI will decrease the ratings of soreness sooner than TWI at 24h-, 48h, and 72h-post muscle-damaging exercise.

_Rationale:_ Previous research has demonstrated decreases in soreness with the use of heating modalities after muscle-damaging resistance exercise (Kim, Monroe, et al., 2020a; Kim, Reid, et al., 2020).

Hypothesis 4: HWI will result in lower levels of pro-inflammatory (CRP, IL-6, TNF-α) and higher levels of anti-inflammatory markers (IL-10) in the blood sooner than TWI at 24-, 48-, and 72h-post muscle-damaging exercise.

_Rationale:_ Previous research has demonstrated decreases in IL-6 and TNF-α utilizing short-wave diathermy after muscle damaging resistance exercise (Vardiman et al., 2015b). (Pournot et al., 2011). Currently, no data exist to suggest that HWI will affect the inflammatory process either negatively or beneficially.

**Scope of the study**

**Subjects for research:** The proposed study was conducted on ten young (18-35), resistance-trained female and male subjects. Participants were included if they engaged in
RT (≥ 2-days/week for ≥ 12 months) which include a variation of leg press, squat, or lower-body RT routine. Furthermore, their sets of lower-body RT typically consisted of 3-10 repetitions per set with moderate-high intensities (75-90% of 1-RM), traditional tempos (e.g., 2-4 seconds per repetition), and some of their sets were performed close to momentary muscular failure. Importantly, they were not currently using thermal recovery strategies post-exercise.

**Study Protocol:** Utilizing a randomized cross-over experimental design, participants were assigned to HWI or TWI experimental sessions upon completion of a muscle-damaging exercise protocol. Venous blood (for the assessment of CK, Mb, CRP, IL-6, IL10, and TNFα) and muscle soreness via a soreness scale were collected prior to muscle-damaging protocol, as well as 24-, 48-, and 72-h post-exercise. Muscle function was assessed by exercise testing, which included vertical jump, and MVC of the quadriceps and occurred 24-, 48-, and 72-h post-exercise.

**Practical applications:** The results from the proposed study can be applied by athletic trainers, personal trainers, and strength & conditioning specialists. For example, an athletic trainer can apply HWI on an athlete after a high-intensity, muscle damaging protocol/practice to potentially accelerate the recovery process. Personal trainers can apply the information from the proposed study to their clients by using it as a recovery strategy in order to increase the number of training sessions they can do at higher volumes. In this way, the client may be able to increase their hypertrophic response. Finally, a strength and conditioning specialist may use post-exercise HWI as an alternative recovery strategy to other approaches, including CWI and light therapy. Especially in season, CWI is used to decrease the inflammatory response and increase
recovery for the next training session. In turn, the adaptation to the training stimulus is blunted. Therefore, the use of heat may have a medium to large effect magnitude on recovery of performance indices such as maximal voluntary isokinetic contraction. The results from the present study provide insight into how HWI may play a role in recovering from muscle damaging resistance exercise.

**Significance**

The effects of heat, both acutely and chronically, are due to increases in core, skin, and local muscle temperature. Increases in environmental heat have been used to increase core temperature, while local heat therapy has been used to increase local tissue (i.e., skeletal muscle) temperature. Similarly, HWI has been used to act as a local heat therapy while increasing core temperature. While HWI has been tested for isometric squat strength and peak power, it has not been tested on other sport related performance such as vertical jump or maximal voluntary isometric and isokinetic contractions. Additionally, the effect of HWI on blood markers of inflammation after a muscle damaging protocol have not been studied. Therefore, the present study provided insights into how a HWI protocol after a session of muscle-damaging resistance exercise can affect recovery of exercise performance and markers of inflammation.

**Assumptions**

This study was conducted based on the following assumptions

1. Participants answered the health history and physical activity questionnaires honestly and followed pre-test guidelines before each of their visits.
2. Participants maintained the same lifestyle routine throughout the duration of their involvement in the study, especially as it pertained to their exercise habits.

3. Participants executed their 1-RM, muscle damaging protocol, vertical jump, and MVC tests with maximal effort.

4. Each repetition was performed with perfect uniformity and the lifter maintained the desired tempo during each condition.

5. All equipment in the study was in good working order, including the thermistor, thermometer, water heating coil, and heart rate monitor.

Limitations

1. External validity – Our data was collected in young, resistance-trained females and males. The results should be applied with caution to populations with different ages, training statuses, and training modalities. Similarly, the HWI will be set to 42° C and will be up to the sternum. This heating protocol may be too uncomfortable for some people and thus may decrease its applicability. Similarly, access to a tub with the ability to raise to 42° C may not be possible.

2. Internal validity – It is important to acknowledge the limitations of the measurements tools that will be used to acquire data for the dependent variables in this study. First, blood CK and Mb provide an indirect and systemic measurement of exercise-induced muscle damage after a muscle damaging protocol, and thus do not perfectly reflect the damage that is occurring at the muscle. Second, inflammatory markers are systemic and will be used to quantify both the anti- and pro-inflammatory response. Inflammatory markers are consistently in flux outside of the muscle. As such, the inflammatory markers may not perfectly reflect the inflammation that is occurring post-exercise at the muscle.
level. Third, exercise testing, while able to quantify losses in strength, is an indirect marker of muscle damage and does not quantify how much damage has occurred.

3. Ecological validity – Although we will attempt to control effort during sessions of resistance exercise, our goal is to elicit high amounts of muscle damage. As such, it is possible that effort may decrease during the session as pain/fatigue begins to set in. When applied to practice, it is possible that a lifter or athlete will use differing rep ranges to elicit a stimulus while mitigating damage. Importantly, muscle damage can happen outside of the weight room with differing modalities, but we chose to use two exercises (leg press and plyometric lunges) for the convenience of data collection and to minimize the number of required repetition-max testing and familiarization sessions.

Abbreviations

EIMD = Exercise-induced muscle damage
MVC = maximal voluntary contraction
DOMS = delayed onset muscle soreness
CK = creatine kinase
LDH = lactate dehydrogenase
ROS = reactive oxygen species
NOS = nitric oxide species
Ca++ = calcium
ATP = adenosine triphosphate
CWI = cold water immersion
WBC = whole body cryotherapy
CNS = central nervous system
mTORC1 = mammalian target of rapamycin complex 1
FOX O1 = forkhead box protein O1
4E-BP1 = Eukaryotic translation initiation factor 4E-binding protein 1
Rps6 = ribosomal protein s6
HWI = hot water immersion
TWI = temperate water immersion
CRP = C-reactive protein
IL-6 = Interleukin-6
IL-10 = Interleukin 10
TNFα = Tumor necrosis factor alpha
Chapter I References


**Chapter II: Literature Review**

This chapter represents a review manuscript entitled “Recovery strategies to mitigate resistance exercise-induced muscle damage in physically active individuals” that has been formatted for publication in International Journal of Sports Medicine. It is authored by Rogelio A. Realzola, Flavio de Castro Magalhaes, Christine Mermier, Michael Deyhle, and Fabiano Amorim. The manuscript follows the formatting and style guidelines of the journal and the references cited are provided at the end of the manuscript.
Recovery Strategies to Mitigate Resistance Exercise-Induced Muscle Damage on Physically Active Individuals

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Running header: Recovery strategies for resistance exercise-induced muscle damage
Abstract

Exercise-induced muscle damage (EIMD) is typically caused by eccentric or unaccustomed exercise. At the cellular level, EIMD can be characterized as structural changes within the sarcomere, increased permeability of the sarcolemma, decrements in action potential propagation, and degradation of contractile proteins. These structural damages result in increased muscle soreness and ratings of perceived exertion, as well as with a decrease in the ability to generate muscle strength and power. Concurrently with the structural damage, an inflammatory process plays an important role in the cellular and function recovery process. The alleviation of symptoms of EIMD may be advantageous to individuals who require rapid recovery between bouts of exercise. As such, understanding the underlying mechanisms of EIMD can help to implement recovery strategies that may ameliorate performance decrements and decrease the time it takes to return to peak performance. In the first section of this article, the mechanisms underlying EIMD associated with resistance exercise-induced muscle damage (REIMD) will be reviewed focusing on the structural, functional, and metabolic level disturbances. In the second section, popular recovery strategies associated with REIMD recovery will be reviewed with a focus on the efficacy of several commonly used pharmacological, nutritional, and heating/cooling interventions.
Introduction

Exercise-induced muscle damage (EIMD) typically occurs when an individual is exposed to unaccustomed exercise, exercise greater than normal intensity or duration, or after eccentric-based exercise [35,75]. EIMD is characterized by structural and functional changes in subcellular structures that can be categorized as indirect or direct markers. Myofibrillar disruption (i.e., z-disk streaming), muscle fiber necrosis, immune cell infiltration into the fibers, and losses of cytoskeletal protein identified by electron microscopy after a bout of exercise are direct signs of EIMD [22,28,35]. Indirect markers of EIMD measured by the release of muscle proteins into the circulation, such as elevated levels of creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (Mb) also serve to indirectly demonstrate that subcellular structures damage has occurred [35].

Further, delayed-onset muscle soreness (DOMS), and decreases in muscle function measured by maximal voluntary contraction (MVC) also provide indirect evidence that EIMD has occurred [35,75]. As such, understanding the mechanisms of EIMD and the recovery process is important for proper implementation of strategies for exercisers and athletes. Several recovery strategies using nutritional and topical components (i.e., CBD and vitamins), and temperature-based modalities (cold and hot) have been proposed to reduce the recovery time and restore performance after an EIMD bout. There remains a paucity of studies on the effects of these recovery strategies specifically on resistance exercise induced-muscle damage (REIMD). Understanding their effects on performance after a muscle damaging bout of exercise is of key importance, especially as athletes and practitioners seek to optimize recovery [40]. The first section of this narrative review will focus on the facets of EIMD, while the second section will describe the current literature.
on recovery strategies for REIMD. By arming athletes and professionals in the field with the information within this review, we hope that they can make informed science-based decisions about which modalities to invest in and how to implement them.

**Overview of EIMD**

EIMD is related to disruptions of subcellular skeletal muscle structures and characterized by loss of muscular function measured by MVC, DOMS, swelling, and/or reduced range of motion. Further, damage to the skeletal muscle membrane is associated with increases in the systemic circulation of intracellular content such as CK, LDH, and Mb [62].

**Mechanical Strain**

The current consensus for the first step in the development of EIMD is the overstretching of myofibrils that occur during muscular contraction [23,35]. EIMD is most often caused by high intensity eccentric exercise. Evidence suggests eccentric exercise decreases motor unit activation when compared to isometric or concentric contractions [35]. The decrease in motor unit activation leads to greater force per active motor unit, causing additional mechanical stress, and more pronounced damage to the sarcomere. This may lead to altered recruitment patterns of motor unit activation due to disruptions in the excitation-contracting coupling process [35].

Specific to the sarcomere, individual sarcomeres in a myofibril experience disproportionate stress given differences in length-tension relationships. This may be due to the actin-myosin overlap being further away from their optimal point or the cross-sectional area being smaller than other sarcomeres [75]. The disproportionate strain
causes the sarcomeres to become weaker, causing the next sarcomere to load at higher rates and weaken as time continues, leading to “popped sarcomeres” [56]. Consequently, this continues until the muscle relaxes or is injured. Upon relaxation, myofilaments of overstretched sarcomeres are re-aligned, but some do not. This misalignment presents itself as z-line streaming, which predominately occurs 24-72h postexercise [28]. Importantly, myofibrils are anchored to the membrane via costamere protein complexes that includes key proteins such as dystrophin. Overstretching can be problematic for the integrity of the extracellular matrix (ECM) and the membrane [75].

Finally, mechanical strain and the subsequent decrease in sarcomere strength, as well as an increase in membrane permeability plays a key role in force loss [35,75]. The mechanical alterations to the cytoskeleton from EIMD stimulate the activation and proliferation of immune cells that initiate tissue repair and remodeling [35], which will be focused on later in this review. Understanding the phases of EIMD is necessary for the implementation of proper recovery strategies.

**Force Loss**

Immediately post-eccentric exercise and in the following days, EIMD presents itself as a decline in muscle force production and is thought to be a more objective indication than other markers such as increased levels of CK, LDH, and soreness [15,16]. Within the first 24-48 hours, force loss can range between 15-60% and can remain for up to 2 weeks depending on the severity of EIMD [35]. Resistance exercise tends to result in more decrements in force loss when compared to EIMD induced by long-duration running, downhill running, or non-weight bearing training modalities [23,35,62,75]. Further, EIMD is greater when exercises are performed at higher eccentric torque,
increased numbers of eccentric muscle contractions, long vs short muscle lengths, and exercise using single joint vs multiple joints [35,62]. A decrease in membrane integrity may be responsible for increases in membrane permeability, which can lead to decrements in excitation-contraction coupling (ECC), and a subsequent decrease in force production via failed ECC [88]. ECC inefficiency is believed to be due to an influx of extracellular calcium which can upregulate calcium-activated calpains that can destroy key contractile and structural proteins such as junctophilins [5]. Increased calcium can also act on calcium-dependent phospholipase A₂ (PLA₂), which stimulates reactive oxygen species (ROS) via release of arachidonic acid [39]. While the effects of increased ROS on membrane integrity remain to be elucidated, ROS are important for optimum levels of isometric force production [72]. During exercise, the rate of O₂⁻ production is increased dependent on intensity and duration of the exercise, as well as the temperature of the contracting muscle. Free arachidonic acid, which is released by PLA₂ cleaving membrane phospholipids, is a substrate for several ROS-generating enzyme systems [92]. Further, activation of PLA₂ can activate NADPH oxidases (NOX), such as NOX2 and NOX4. NOX4 is known to contribute to the basal rate of ROS production in muscle fibers, while NOX2 is the primary source of NOX mediated ROS production in contracting muscle [87]. Indeed, contracting muscle, and specifically NOX within the contracting muscle, are thought to be the dominant source of ROS production during exercise [39]. While ROS are necessary for optimum force production, there exists a biphasic relationship, such that high levels of ROS results in decrements of force production [70]. Similarly, it is possible that ROS-mediated decreases in Na⁺/K⁺ pump also contribute to decreases in force production. Importantly, perturbations in force
production are generally studied within the context of endurance exercise, and the relationship between ROS and force output during resistance exercise remains unclear. Still, it is clear that removal of O2\(^{-}\) via superoxide dismutase (SOD) decreases maximal force production, while increased ROS levels in the fiber above the optimal point results in force generation [71]. While force loss is an index of EIMD, the inflammatory response plays a crucial role in EIMD, especially after eccentric exercise [80].

**Inflammatory Response**

Eccentric exercise and muscle damaging necessitate an ensuing inflammatory response that may contribute to exacerbating EIMD [14,27,35,82]. Likewise, even small amounts of damage, either through passive stretching or isometric loading, can increase the neutrophil infiltration [65,66]. Importantly, mast cells, thought to be the first to respond to a muscle damaging insult, are responsible for detection of tissue injury and initiate early leukocyte and macrophage recruitment via the release of histamine and chemoattractants (Côte et al., 2008). Indeed, the concept of neutrophil invasion being the first step in the inflammatory response is challenged by increased macrophage infiltration after EIMD without a preceding neutrophil invasion [19]. Still, the importance of neutrophils in the inflammatory response cannot be overlooked. It is likely that the chemoattractant for macrophages differs from those of neutrophils and is released at a later point. In addition to neutrophils and macrophages, mast cells, T regulatory lymphocytes, eosinophils, and CD8 T lymphocytes are immune cells that are central to the recovery process [14]. In the early hours of recovery, neutrophils are the predominant inflammatory cell that act to clear cellular debris and increase macrophage infiltration via secretion of cytokines [14,27,82]. Consequently, this can happen with resistance exercise,
downhill running, and long distance running [47–49]. Importantly, evidence suggests that neutrophil concentration diminishes after 24 hours and likely do not play a role past this time-point [14,27,82]. Between 4 – 24 hours after damage, macrophages invade muscle and work phagocytizing damaged tissue, initiating myoblast proliferation, and secreting proinflammatory cytokines [14,78,82]. This increase in macrophages is driven by T helper type 1 (Th1) cytokines such as interferon-γ (IFN-γ) and TNF-α which drive activation of macrophages to an M1 phenotype [82]. M1 macrophages are responsible for secreting pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6, as well as leukocyte protease inhibitor [62]. Cytokine production is responsible for degradation of damaged muscle and muscle remodeling through increased gene expression of muscle ring finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) [57]. Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor that is present in the cytosol in an inactive form, is also activated via elevated cytokines. Upon activation, NF-κB can dissociate from its inhibitor and translocate to the cell nucleus and induce transcription of iNOS, TNF-α, and IL-1, which serves to increase expression of inflammatory mediators like C-C Motif Chemokine Ligand 2 (CCL2) and IL-6 [82]. Importantly, NF-κB can promote cell proliferation and inhibition of differentiation, via promotion of cyclin D1 in muscle, and destabilization of MyoD mRNA, respectively [82]. Notably, M1 macrophages play a pro-inflammatory role for approximately 2 days, after which Th2 cytokines such as IL-4, IL-10, and IL-13, shift them to an M2 phenotype. The M1 to M2 polarization is mediated by STAT protein activity. Specifically, STAT6 is activated by IL-4 and/or IL-3, and is required for M2 polarization [58]. M1 macrophages, which are characterized by a
strong expression of CD163, are important in repair and remodeling via activation of satellite cells. M2 macrophages attenuate inflammation, stimulate satellite cell differentiation, and promote muscle regeneration [14,27,58,82]. Thus, the inflammatory response is a tightly regulated process responsible for effectively stimulating regeneration of muscle via a calculated influx of pro- and anti-inflammatory cytokines and macrophages. It is postulated that the inflammatory process plays a key role in the development of muscle soreness [35].

**Delayed Onset Muscle Soreness**

Delayed onset muscle soreness (DOMS) is the soreness that presents itself within 24-48 hours post-exercise [35]. While the extent of DOMS does not correlate well with muscle damage observed in muscle biopsies, it does correlate well with high levels of volume load, and eccentric muscle contractions [35,75]. DOMS is believed to be due to a combination of muscle damage, disruptions in Ca\(^{2+}\) homeostasis, and activation and subsequent hypersensitivity of nociceptors within the ECM [35,54]. In fact, data has suggested that the inflammatory process plays a more pronounced role in DOMS than actual muscle damage. (Chazaud, 2016; Le Moal et al., 2017). Further, DOMS may be more associated with inflammation within the ECM as opposed to myofiber damage and inflammation [21]. Muscle damage produced by eccentric exercise upregulates CCL2, upregulating infiltration of macrophages [24]. The subsequent accumulation of macrophages may lead to a release of histamines, bradykinin (BK), and prostaglandins (PG) [35]. Although not fully understood, the release of BK and PG are thought to stimulate creation of nerve growth factor (NGF) via activation of the B\(_2\)-BK receptor-nerve growth factor pathway [54]. Similarly, activation of the cyclooxygenase (COX)-2
glial cell line-derived neurotrophic factor pathway, produced by macrophages and satellite cells, may induce DOMS via hypersensitivity of nociceptors [54]. It should be noted though, that Mizumura et al. (2016) have shown that macrophages are not required for BK and PG release, and evidence suggests that BK and PG production occurs during or immediately after exercise, thus decreasing the importance of macrophages on this specific pathway. Finally, nociceptor stimulation may also be due to secretion of neurotrophins from muscle fibers[35], although these mechanisms are less understood.

Recovery strategies

Recovery after a session of exercise training and sport training/competition refers to the physiological aspect of recovery, which is preceded by physical fatigue induced by training or competition [40]. Further, “recovery” is an umbrella term that can be characterized by different modalities of recovery. Indeed, the use of active recovery strategies (like taking supplements) and passive recovery strategies (thermal) may help to facilitate the process. REIMD has been used to assess the recovery period required between resistance exercise training sessions. As eccentric resistance exercise may produce greater muscle damage compared to concentric and non-resistance exercise training [9], it is important to understand the potential benefits and drawbacks of popular recovery modalities and their physiological processes. In this way, athletes and practitioners will be able to more effectively choose multiple strategies to facilitate improvements in REIMD. Below, we describe popular recovery strategies that are proposed to aid in REIMD.

Supplements
Numerous nutrition and supplemental recovery strategies have been studied for their potential to ameliorate EIMD. Most notably, curcumin, tart cherry juice, and cannabidiol (CBD) and their effects on EIMD have been studied with varying results. Exercise scientists and athletes have identified these supplements as solutions that are practical, easy to use, and may not have potential decrements with long-term use. These substances are proposed to work as anti-inflammatory and/or antioxidants, as well as potentially modulating transcription factors for muscular hypertrophy [36,37,77]. Supplementation can possibly create subtle changes with superior adaptations, especially when nutrition, sleep, load management, and stress are accounted for. This section of the review will focus on the evidence derived from emerging supplements used to recover from EIMD, with particular reference to supplements that have possible positive effects on REIMD.

**Curcumin**

Curcumin is a polyphenolic substance extracted from turmeric, and is proposed to work as an anti-inflammatory and antioxidant through a decrease in the expression of pro-inflammatory genes [76]. Importantly, curcumin acts by attenuating neutrophil migration and lowering ROS production [76]. Data on humans and what transpires at the cellular level is lacking, but animal models have given insight to physiological processes. Curcumin has been shown to inhibit macrophage infiltration by attenuated CCL2 expression at the muscle, as well as inhibiting ROS production [76]. A decrease in macrophage infiltration would theoretically decrease DOMS via a decrease in BK and PG expression, limiting the increase in NGF and/or glial-cell derived neurotrophic factors.
(GDNF) that would hypersensitize nociceptors (Hyldahl & Hubal, 2014). Similarly, curcumin can regulate inflammatory cascades of NF-κB and NF-E2 related factor 2(Nrf2) pathways, which may also aid in the reduction in hypersensitization of nociceptors [78,82]. Currently, few studies have investigated the effects of curcumin on REIMD (Table 1). Regarding soreness, evidence that curcumin aids in DOMS is mixed. Tanabe et al. (2019) has shown that .09 mg of curcumin supplementation twice daily for 7 days prior to eccentric resistance exercise, and 4-7 days post-eccentric resistance exercise showed decreases in soreness when compared to placebo for maximal isokinetic eccentric contractions of the elbow flexors, and isometric MVC of the elbow [77,79]. Similarly, Basham et al. (2019) showed decreases in soreness after 225 single leg sit-to-stand when supplemented with 1.5g of curcumin for 28 days [3]. Importantly, Nicol et at. (2015) and McFarlin et al. (2016) observed no difference between curcumin treatment and placebo [51,59]. The lack of differences in soreness between the studies cited above may be due to different supplementation strategy, as participants only received 25 g of curcumin 2.5 days pre- and post-exercise [59], and 0.4 mg 2 days pre- and 3 days-post [51] before resistance exercise. Further, the resistance exercise modality used to induce muscle damage involved the use of eccentric leg press in both studies, which differed from previous curcumin studies [51,59]. While more data is needed, the current evidence suggests that curcumin, when dosed appropriately, can help to alleviate symptoms of DOMS.

Regarding performance markers after a bout of resistance exercise, the effects of curcumin are less clear. Curcumin supplementation has shown attenuation of decrements in muscle strength [77], and range of motion [77,79]. Still, Nicol et al. (2015) observed
no changes in single leg jump. Given that there are fewer studies examining performance markers of EIMD, it is difficult to say whether curcumin aids in performance recovery. Although yet to be fully understood, it is proposed that curcumin acts to decrease NF-κB, a positive regulator of iNOS. An increase in iNOS can increase NO and peroxynitrite (ONOO'), both of which may induce muscle membrane lysis, negatively impacting the ability for muscle to contract [35,82]. Finally, the effects of curcumin supplementation on blood markers of damage and inflammation have been studied with mixed results [3,51,77]. Curcumin is known to suppress NF-κB and promote muscle regeneration, which would in theory decrease serum CK. Although CK increases post-resistance exercise, the variance in CK values across studies may be due to changes in time-point measurements, influenced by heredity, and training history [77]. Further, markers of inflammation and how they are affected by curcumin have yet to be understood. Basham et al. (2019) observed no change in malondialdehyde, total antioxidant concentration, and TNFα after an eccentric protocol of 225 single leg sit to stands. Results are in agreement with McFarlin et al. (2016), who observed no change in IL-6 after an eccentric-based leg press muscle damaging protocol. Importantly though, McFarlin et al. (2016) showed reductions in IL-8, and TNFα, further confounding the effects of curcumin on inflammation. It is proposed that the reduction in inflammatory markers is due to a reduced PG from decreased COX-2 signaling [51]. Still, differences in results indicate that other pathways may be important for modulation of inflammation. Given the current research, it is clear that further studies are needed to assess timing and dosage strategies, as well as its effects after different resistance training modalities, but data suggests that curcumin can aid in ameliorating EIMD and DOMS.
Table 1. Curcumin interventions for recovery from resistance-exercise induced muscle damage

<table>
<thead>
<tr>
<th>Study</th>
<th>Resistance training protocol</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basham et al., 2019</td>
<td>225 single leg sit</td>
<td>20 trained males</td>
<td>3 x 0.5g capsules for 28 days</td>
<td>↔MDA, TAC, TNFα ↓ CK, Soreness</td>
</tr>
<tr>
<td>McFarlin et al., 2016</td>
<td>6 x 10 leg press with 5-s ECC@ 110 1RM</td>
<td>28 resistance trained males and females</td>
<td>0.4g curcumin supplement 2 days pre-, 3 days post-exercise</td>
<td>↔IL-6, IL-10, Soreness ↓ CK, IL-8, TNFα</td>
</tr>
<tr>
<td>Nicol et al., 2015</td>
<td>7x10 ECC single-leg leg press repetitions</td>
<td>17 males</td>
<td>25g of curcumin (5 2.5g capsules 2x daily after exercise) 2.5 days pre and post exercise</td>
<td>↔Single leg Jump, Soreness, IL-6, TNFα</td>
</tr>
<tr>
<td>Tanabe et al., 2019</td>
<td>30 maximal ISOK ECC contractions of elbow flexors</td>
<td>24 healthy men</td>
<td>0.09g curcumin supplement 2x Pre – 7 day before, Post – 4 day post</td>
<td>↓ ROM decrements, Soreness</td>
</tr>
<tr>
<td>Tanabe et al., 2018</td>
<td>3 5-s MVC at 90 degrees</td>
<td>10 healthy men</td>
<td>0.09g curcumin supplement Pre – 7 days before, 24h before, Post -7 days post</td>
<td>↓ Decrements in muscle strength, ROM, soreness, CK</td>
</tr>
</tbody>
</table>

ECC = eccentric, 1RM = 1-repetition maximum, ISOK = isokinetic contraction, MVC = maximal voluntary contraction, CK = creatine kinase, MDA = Malondialdehyde, TAC = Total antioxidant capacity, TNFα = tumor necrosis factor alpha, IL-8 = Interleukin-8, IL-6 = Interleukin-6, IL-10 = Interleukin-10, ROM = range of motion
**Tart Cherry Juice**

Tart cherry juice from Montmorency cherries contains phytochemicals, namely anthocyanins and flavonoids [63]. Anthocyanins, which have a high antioxidant content, are theorized to be effective in limiting ROS production by scavenging ROS [33,44,63]. Anthocyanins are known to inhibit Th2 cytokines (IL-13) and pro-inflammatory cytokines (IL-6 and TNF-α) in a murine asthma model [61]. Further, anthocyanins can inhibit cyclooxygenase-2 (COX-2) and phospholipase A2, potentially leading to limitations in neutrophil accumulation and mediation of pro-inflammatory cytokines [50,62,82]. Anthocyanins may also inhibit NF-kB related pro-inflammatory cytokines in in vivo and in vitro models, suggesting that they play an anti-inflammatory role [43,50]. This may be further exemplified by significant decrease in plasma TNF-α and IL-6 [50]. Indeed, a decrease in T helper type 2 (Th2) cytokines may decrease pro-inflammatory cytokine release and subsequent macrophage proliferation, but the data on tart cherry, especially within the context of REIMD, remains questionable.

To our knowledge, only a handful of studies have sought to quantify differences in performance, soreness, and inflammatory markers with tart cherry supplementation and REIMD (Table 2). Previous research has found that the use of tart cherry has been beneficial for MVC, counter-movement jump (CMJ), 20 m sprint, and agility performance, especially 72h post-exercise [6]. It is important to note that the applicability to resistance training is unclear, as Bell et al. (2016) utilized a shuttle test to mimic intermittent exercise. In the context of marathon running, while there were no differences between tart cherry and placebo for CK, LDH, or muscle soreness, there were significant
differences in IL-6 and total antioxidant capacity [34]. Specific to resistance training, studies have shown mixed results in the efficacy of tart cherry. In untrained men, it seems that tart cherry juice did not have a positive effect on EIMD, given that no differences were observed in MIVC, DOMS, range of motion (ROM), and CK [44]. These findings are corroborated by studies utilizing trained men, which found no differences in soreness, ROM, thigh circumference, isokinetic strength, CK, or IL-6, IL-8, and TNFα repetitive isokinetic maximal voluntary [4]. While Lamb et al. (2019) used 30 mL of concentrate, Beals et al. (2017) dosed participants with 2 servings of 60 g tart cherry powder for 4 days pre- and 8 days post-exercise. Thus, dosing strategies, although different, yielded similar results in trained and untrained individuals. Specific to soreness, Connolly et al. (2006) is the only group who observed a decrease in pain for their tart cherry supplement group compared to placebo. Importantly, although there was a decrease in soreness, there was no change in muscle tenderness, which can arguably be considered to be soreness [18]. These data are similar to other studies which show no change in soreness regardless of dosing and different resistance exercise protocols [8,33,44,86]. Although few benefits for soreness have been shown with the use of tart cherry juice, supplementation may be beneficial for MVC recovery [8,86] and decreased strength loss after a muscle damaging resistance exercise protocol [18]. Differences in MVC force reduction in the tart cherry juice groups is proposed to be due to altered Ca\textsuperscript{2+} sensitivity via glutathione binding by oxidized troponin I cysteine residues [86]. Further, glutathione peroxidase (GPX) enzymes catalyze the reduction of H\textsubscript{2}O\textsubscript{2} and organic hydroperoxides by glutathione, which may contribute to attenuation of MVC force loss [86]. These data are interesting, as another study found that tart cherry reduced oxidative stress, CK, CK-MB (cardiac
muscle CK isoform), and attenuated reductions in grip strength following a bout of intense resistance exercise, while no changes in jump power were present [33]. The confounding effects of tart cherry may be due to differences in dosing strategies. Specifically, Hooper et al. (2021) loaded 0.5 mg of tart cherry for 7 days, while Lamb et al. (2019) had participants consume 2 x 250 mL of tart cherry daily for nine days, and Wangdi et al. (2021) supplemented participants with 2 x 30 mL of Montmorency cherry concentrate for 10 days. Thus, the data on tart cherry juice on CK and MVC are still unclear.

Although tart cherry juice is believed to be beneficial for inflammation because of anthocyanins, the data on its effects on REIMD are mixed. As previously mentioned, Beals et al. (2016) observed no difference in IL-6, IL-8, or TNFα after isokinetic contractions until failure. These results are supported by a study showing no differences in Nitrotyrosene, CRP, and total antioxidant status after eccentric knee extensions [8]. While tart cherry juice may not have an effect directly on inflammatory markers, there does seem to be a benefit in antioxidant markers. Importantly, Bowtell et al. (2011) did show a decrease in protein carbonyls, while Wangdi et al. (2021) observed increased superoxide dismutase 3 (SOD3), GPX3, and GPX4 mRNA, which may play a role in increasing recovery. Ultimately, tart cherry remains a viable, but perhaps unreliable method for ameliorating indices of EIMD. Further studies are needed to assess dosing strategies, timing, and its effects on various modalities [53].
Table 2. Tart Cherry Juice interventions for recovery from resistance-exercise induced muscle damage

<table>
<thead>
<tr>
<th>Study</th>
<th>Resistance training protocol</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beals et al., 2016</td>
<td>Repetitive ISOK at 60°/s until fatigue</td>
<td>29 recreationally active males &amp; females</td>
<td>2 servings of 60g TartVitaCherry® powder 4 days pre-, and 8 days post-exercise</td>
<td>↔ Soreness, ROM, Thigh circumference, ISOK strength, IL-6, IL-8, TNFα, CK</td>
</tr>
<tr>
<td>Bowtell et al., 2011</td>
<td>10 x 10 single leg ECC knee extensions at 80% 1RM</td>
<td>10 resistance trained males</td>
<td>30mL 2x/day for 10 days of Montmorency cherry juice concentrate (CherryActive®)</td>
<td>↑MVC recovery ↔ Soreness, Nitrotyrosene, TAS, CRP ↓CK, PC</td>
</tr>
<tr>
<td>Connolly et al., 2006</td>
<td>2 x 20 ECC contractions of the elbow flexors</td>
<td>14 college aged males</td>
<td>~355mL freshly prepared tart cherry juice, 2x/day, 3 days pre-, 4 days post</td>
<td>↔ Motion Loss, Muscle Tenderness ↓ Strength Loss, Pain</td>
</tr>
<tr>
<td>Hooper et al., 2021</td>
<td>6x10 80% back squat</td>
<td>13 resistance trained men</td>
<td>0.5g broad spectrum of polyphenols</td>
<td>↔ Jump Power, Soreness ↓ PC, Handgrip Strength, CK, CKMB</td>
</tr>
<tr>
<td>Lamb et al., 2019</td>
<td>5x10 ECC MVC elbow flexors</td>
<td>36 non-resistance trained men</td>
<td>30mL concentrate diluted with 220 mL of water</td>
<td>↔MVC, Soreness, ROM, CK</td>
</tr>
<tr>
<td>Wangdi et al., 2021</td>
<td>10 x 30 maximal ECC knee flexion contractions</td>
<td>10 recreationally active males</td>
<td>2 x 30mL daily, for 10 days of Montmorency</td>
<td>↑ MVC recovery, SOD3, GPX3, GPX4 mRNA</td>
</tr>
</tbody>
</table>
Cannabidiol

Recently, the use of cannabidiol (CBD) as a recovery strategy has increased dramatically [10]. CBD is a non-euphoric compound of marijuana that, unlike THC, does not activate cannabidiol receptor 1 (CBR1) and thus has no psychoactive properties [10]. Importantly, while it does not invoke euphoria, it has been postulated to act as an anti-inflammatory as well as playing a key role in the muscle differentiation process [36,37]. CBD may play a role in CBR activation through indirect methods. CBD may act on cannabidiol receptor 2 (CBR2), which functions to inhibit fatty acid amide hydrolase (FAAH). Importantly, FAAH degrades anandamide (AEA), which is the most abundant endocannabinoid in the body. With the downregulation of FAAH, it is proposed AEA concentrations increase in the brain and plasma, increasing activation of CBR2 [10]. Activation of CBR2 decreases IL-6, TNF-α, COX-2, and iNOS through A2A receptors. In this way, CBD can protect cells from inflammatory damage. Further, decreased activity of COX-2 can decrease nociceptor hypersensitivity due to decreased PG release, potentially decreasing DOMS. Decreases in DOMS have been shown in previous studies, albeit in rat models [31]. It has also been postulated that CBD may decrease release of cytokines through NF-kB pathways, although the specific processes remain unclear [10,36]. Finally, the potential for CBD and its effect on muscle differentiation processes

<table>
<thead>
<tr>
<th>cherry concentrate</th>
<th>↔ Jump Height, Soreness</th>
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<tbody>
<tr>
<td>ISOK = isokinetic contraction, ECC = eccentric, MVC = maximal voluntary contraction, ROM = range of motion, IL-6 = Interleukin-6, IL-8 = Interleukin-8, TNFα = tumor necrosis factor alpha, CK = creatine kinase, PC = protein carbonyls, TAS = total antioxidant status, CRP = C-reactive protein, CKMB = creatine kinase-MB, SOD3 = superoxide dismutase 3, GPX3 = glutathione peroxidase 3, GPX4 = glutathione peroxidase 4</td>
<td></td>
</tr>
</tbody>
</table>
It is postulated that CBD may do the following: increase dystrophin, increase differentiation of human myoblasts into myotubes, increase differentiation of human muscle satellite cells into myotubes, and ameliorate impaired locomotor activity and muscle strength [37].

While the data on CBD and its effects on EIMD in mice and cells seem enticing, data on humans and how CBD affects REIMD does not seem promising. Presently, the few studies that were done to assess CBD and its effects on EIMD after a resistance exercise protocol have shown mixed results. With 1ml dose of CBD oil containing approximately 16.67 mg of CBD and medium-chain triglyceride (MCT), CBD significantly decreased DOMS at the 0-24, and 48-72 hour time points when compared to placebo [32]. On the other hand, 2.0 mg/kg (approximately 150mg) of CBD in a pill was shown to have no effect on muscle soreness, inflammation, and strength performance after a damaging bout of eccentric resistance exercise [17]. Crossland et al. (2022) showed similar results with 5mg/kg CBD 10h post-exercise after a damaging eccentric leg extension protocol, demonstrating no change in IL-6, IL-10, IL-1β, dynamic strength, soreness, and Mb when compared to a placebo group [20]. Although Isenmann et al. (2021) also observed no difference in counter movement jump, there were significant decreases in CK and Mb with CBD after a damaging squat and drop jump protocol. It is our belief that the lack of consensus on CBD and its effects on REIMD are due to differences in dosage strategies (pills vs oil vs oil mixed with other transports), application (ingestion vs topical cream), timing strategies, and damaging protocols. Clearly CBD has not been well-studied, and there is a lack of consensus on the efficacy
of it as a recovery strategy. It remains to be seen what effects, if any, CBD may have on different markers of EIMD with various dosing strategies and modalities.
Table 3. Cannabidiol interventions for recovery from resistance-exercise induced muscle damage

<table>
<thead>
<tr>
<th>Study</th>
<th>Resistance training protocol</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cochrane-Snyman et al., 2021</td>
<td>6 x 10 ECC ISOK contractions of elbow flexors at 30°/s</td>
<td>13 untrained men</td>
<td>2 x 75mg separated by 8 hours</td>
<td>↔ Arm circumference, Peak torque, Soreness</td>
</tr>
<tr>
<td>Crossland et al., 2022</td>
<td>10 x 10 ECC leg extensions at 30°/s</td>
<td>27 female Division I &amp; II athletes</td>
<td>5 mg/kg CBD isolate pill 10h post-exercise</td>
<td>↔ IL-6, IL-10, IL-1β, Dynamic strength, Mb Soreness</td>
</tr>
<tr>
<td>Hattchet et al., 2020</td>
<td>4 x 10 squats at 80% 1RM</td>
<td>23 participants</td>
<td>1 ml CBD/MCT oil containing 16.67 mg CBD</td>
<td>↓ Soreness</td>
</tr>
<tr>
<td>Isenmann et al., 2021</td>
<td>3 x 12 squats at 70% 1RM, 3 x 15 drop jumps</td>
<td>16 participants</td>
<td>60mg soluble CBD in 250ml water immediately post-exercise</td>
<td>↔ CMJ ↓ CK, Mb</td>
</tr>
</tbody>
</table>

ECC = eccentric, ISOK = isokinetic, 1RM = 1 repetition maximum, IL-6 = Interleukin-6, IL-10 = Interleukin-10, IL-1β = Interleukin-1beta, Mb = myoglobin, CMJ = countermovement jump, CBD = cannabidiol, MCT = medium chain triglyceride

**Thermal Passive Recovery Strategies**

A lack of consensus exists on how to prescribe thermal strategies to improve recovery and mitigate EIMD. This is due to emerging data suggesting that short-term recovery processes may be attenuating long-term adaptations, specifically with the use of cold strategies [12,30]. Below we discuss the proposed mechanisms whereby cold and heat therapy may affect EIMD.
Cold

Cryotherapy, which describes therapeutic processes involving cold temperatures exposure, is popular as a recovery modality [40,81]. Whether cold water immersion (CWI), whole-body cryotherapy (WBC), or localized cooling (LC), the use of cold to expedite the recovery process has been studied thoroughly [1,7,13,26]. The main aim of cryotherapy is to reduce body core temperature or deep muscle temperature, which lessens perturbations in CNS fatigue, reduces cardiovascular strain, stimulates vasoconstriction, and decreases muscle soreness and swelling [38]. Importantly, while cryotherapy can increase acute performance recovery, there are potential decrements in long-term adaptations to training [81]. It is likely that both increase in performance and decreases in adaptation are due to vasoconstriction impacting leukocyte trafficking and activity [1,38].

Positively, the reduction in macrophage trafficking, which normally causes a release of BK and PG, is mitigated. A depression in BK and PGs may lead to a subsequent decrease in NGF and GDNF, which would lead to less muscle soreness via decreased nociceptor hypersensitivity [35]. On the other hand, previous research observed increases in NGF and GDNF without macrophage infiltration, meaning conclusions about potential benefits should be made cautiously [54]. Soreness may be decreased by inhibition of metabolite sensitive group III and IV muscle afferents, which are sensitive to increases in lactate, hydrogen, and ATP [68]. The decrease in DOMS would allow for increases in ROM and increases in perceptual readiness.

Negatively, the lack of skeletal muscle of macrophage accumulation can lead to decrements in repair and remodeling of skeletal muscle via impacted activation of
satellite cells and failed clearance of necrotic debris [14]. However, considering that typical voluntary contraction-induced muscle damage does not result in damage to the extent of myofiber necrosis and, therefore, does not require extensive clearance of necrotic debris and satellite cell activity leading to de novo myogenesis [23,45], the carryover of these literature on muscle regeneration to muscle repair following contraction-induced damage should be considered with caution. M1 macrophages are responsible for the secretion of TNF-α, IL-1β, IL-6, and IGF-1 [46] a decrease in the amount of pro-inflammatory cytokines is expected. Theoretically, less pro-inflammatory cytokines would mean less degradation of damaged muscle via NF-κB, leading to a subsequent decrease in MuRF1 and MAFbx as well [57]. Although no human data exists in respect to CWI and muscle NF-κB, it is possible that the ensuing decrease in NF-κB would also limit macrophage proliferation via a feed-forward system that increases the expression of inflammatory mediators CCL2 and IL-6 [82]. The proposed suppression of M1 macrophage proliferation may inhibit subsequent satellite cell proliferation, and a consequent reduction in M2 macrophages may decrease satellite cell differentiation, although data examining this process is unclear [27,82]. The decrease in macrophage proliferation has been postulated to be the main culprit of decrements in hypertrophy, as explained by the delaying or suppression of satellite cells and kinases during recovery [74]. Similarly, cold shock proteins inhibit satellite cells from differentiating by decreasing expression of myogenin [74]. Lastly, there is evidence that CWI may lead to protein degradation via increased levels of FOX-O1 protein content, although this data is less understood [30].
Indeed, cryotherapy for the use of recovery from EIMD has been studied extensively, and its positive effects have been upheld by various studies. Within the context of REIMD though, the data seem to be more complex. Studies relating to cryotherapy and recovery from resistance exercise can be found in Table 4.

Curiously, soreness was measured in few studies on REIMD. Two studies, Argus et al. (2017) and Pointon et al. (2011), showed no change in soreness when compared to control [2,67]. Importantly, Argus et al. (2017) utilized CWI at 15° C for 14 min, while Pointon et al. (2011) utilized LC in the form of an ice cuff placed on the quadricep for 20 minutes. Both of those studies showed no difference in MVC, CMJ, or voluntary electromyography (EMG). On the other hand, Wilson et al. (2019) observed decreases in soreness when compared to control utilizing a CWI at 10° C for 10 minutes. Although similar to the previous studies, there was no change in maximal strength, and similar responses in cytokines. The difference in soreness measurements between studies may be due to differences between local and whole-body cooling, as well as a placebo effect [90].

Measurements of strength, maximal force production, and MVC also show mixed results, with multiple studies showing the effects of cryotherapy to be equivocal, and in some cases deleterious [2,29,30,60,67,69,74,85,90,91]. Indeed, only few studies show an increase in MVC recovery [73] and fatigue resistance [91]. The benefits of cryotherapy on recovery are likely due to the intervention being CWI [73,91], thus having a larger effect magnitude of effect than local cooling. These data are made even more inconclusive when comparing differences between acute and chronic cryotherapy, which may play a role at attenuating strength, and hypertrophy [13].
Although cryotherapy has been studied for its effects on the inflammatory response, to our knowledge, only one study has examined it after REIMD. Wilson et al. (2019) used CWI for 10°C for 10 minutes and observed no significant difference for IL-6, CRP, and TNFα. It is believed that the lack of effect on cytokines and markers of inflammation is due to the early and acute rise in markers being unaffected by short or prolonged cryotherapy [90]. Although the use of cryotherapy has been studied for its effects on recovery indices, it is clear that it either generally does not help with REIMD or that the difference in interventions in the presented studies is too great to be able to draw a conclusion.


<table>
<thead>
<tr>
<th>Study</th>
<th>Resistance training protocol</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frohlich et al., 2014</td>
<td>3 x 8-12 CONC and ECC knee flexion at 75-80% 1RM for 2 days/week for 5 weeks</td>
<td>17 recreationally trained males</td>
<td>Immediately post-exercise: CWI: 3 x 4 min at 12°C with 30s rest</td>
<td>↔ Maximal Strength, Squat Jump, Push Up ↓ CMJ</td>
</tr>
<tr>
<td>Fyfe et al., 2019</td>
<td>3 x 12 RM and 20 RM for dips and ab curls for 3 days/week for 7 weeks.</td>
<td>16 recreationally trained males</td>
<td>Immediately post-exercise: CWI: 10°C for 15 min CON: 15 min passive rest</td>
<td>↔ Bench and Leg Press ↓ Fatigue Resistance</td>
</tr>
<tr>
<td>Ohnishi et al., 2004</td>
<td>3 x 8 RM handgrip exercise for 3 days/week for 6 weeks</td>
<td>16 recreationally trained males</td>
<td>Immediately post-exercise: Local CWI: 10°C for 20 min CON: Passive rest</td>
<td>↔ Maximal Strength ↓ Fatigue Resistance</td>
</tr>
<tr>
<td>Poppendieck et al., 2020</td>
<td>3 x 10 RM leg press, knee flexion, and knee extension for 3 days /week for 8 weeks</td>
<td>11 recreationally trained males and females</td>
<td>Immediately post-exercise: CWI: 14-15°C for 10 min CON: 10 min passive rest immediately post-exercise each session</td>
<td>↔ Maximal strength, CMJ</td>
</tr>
<tr>
<td>Yamane et al., 2015</td>
<td>5 x 8 wrist flexion at 70-80% 1RM, 3 days /week for 6 weeks</td>
<td>14 recreationally trained males</td>
<td>Immediately post-exercise: Local CWI: 10°C for 20 min CON: Passive</td>
<td>↓ Maximal Strength, Fatigue Resistance</td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Exercise Protocol</td>
<td>Participants</td>
<td>Immediate Post-exercise Protocol</td>
<td>Recovery Effect</td>
</tr>
<tr>
<td>-------------</td>
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</tr>
<tr>
<td>Argus et al., 2017</td>
<td>3 x 5 deadlifts at 6RM + 3 x 10 back squats, bench press, barbell lunge, and barbell bent over rows at 11RM</td>
<td>13 recreationally trained males</td>
<td>Immediately post-exercise: CWI 15 °C for 14 min CWT: 1 min at 38 °C and 1 min at 14 °C CON: 20 min 23 °C</td>
<td>↔ MVC, CMJ, Soreness</td>
</tr>
<tr>
<td>Pointon et al., 2011</td>
<td>6 x 25 maximal CONC (60°/s)/ECC (120°/s) single leg ISOK knee extension</td>
<td>10 recreationally trained males</td>
<td>Immediately post-exercise: Ice cuff for 20 min CON: 20 min passive rest</td>
<td>↔ MVIC, Voluntary Activation, Voluntary EMG, M-wave amplitude, Soreness</td>
</tr>
<tr>
<td>Roberts et al., 2015</td>
<td>10 x 20 maximal ISOK CONC knee extensions at 90°/s</td>
<td>10 recreationally trained males</td>
<td>Immediately post-exercise: CWI: 10° C for 10 min CON: active recovery cycling at 41 W for 10 min</td>
<td>↑MVC recovery ↔ Fatigue recovery</td>
</tr>
<tr>
<td>Roberts et al., 2015</td>
<td>3-6 x 8 – 12 at 8-12RM for leg press, knee extension, knee flexion 3 x 10-18 walking lunges, plyo jumps</td>
<td>24 recreationally trained males</td>
<td>Immediately post-exercise: CWI: 10° C for 10 min Con: 10 min cycling at ~60W</td>
<td>↓ Maximal Force, Fatigue Resistance</td>
</tr>
<tr>
<td>Vieira et al., 2015</td>
<td>6 x 10 maximal CONC (60°/s) and ECC (180°/s) knee extensions</td>
<td>12 recreationally trained males</td>
<td>Immediately post-exercise: Whole-body cryotherapy: -110° C for 3 min</td>
<td>↔ Jump Height, Power</td>
</tr>
</tbody>
</table>
Heat

The use of heat as a recovery tool has been studied less than the use of cold interventions. Presently, very few data exist to conclusively state whether post-exercise heat treatment is a viable option for recovery and/or decreasing symptoms of REIMD. The proposed mechanism for heat is likely due to the role played on the vasculature in response to increases in temperature, as well as an increase in skeletal muscle temperature [42]. Increased blood flow, heart rate, cardiac output, and decrease in peripheral resistance associated with heat act to remove and enhance transportation of metabolic byproducts away from the active tissue and modulate cellular healing and substrate resynthesis [52,89]. In theory, an increase in vasodilation could mean an increase in immune cells and infiltration accumulation, although to our knowledge, no data supporting this presently exists. Acutely, this may act to speed up repair of damages in musculature, and, in theory, increase the pro-inflammatory response by accelerating macrophage processes. It would stand to reason that an increase in macrophages would lead to a more pronounced M1 macrophage, inflammatory stage. Further, an increase in
Macrophage may mean an increase in anti-inflammatory cytokines IL-6, IL-4, and IL-10 [14,58,82]. Thus, an increased macrophage population may lead to a more pronounced satellite cell proliferation and muscle regeneration. Although these mechanisms are proposed, there is little data to support them in the context of REIMD (Table 5). Currently, a majority of the work on REIMD has been examined through local heating [12,41,64,84], with only one, to our knowledge, involving whole body HWI [83].

The effect of heat therapy on soreness is unclear. With prolonged local heating (8 hours immediately post-exercise), Petrofsky et al. (2017) observed a decrease in soreness. Importantly, this was only present when done immediately, and not 24 hours post-exercise. On the other hand, different heating strategies utilizing local hot water at 54-55° C post resistance exercise [41], HWI for 14 minutes at 38° C post-resistance exercise [83], and short-wave diathermy for 15 minutes pre-exercise [12] have shown no effect in soreness. The difference in soreness may be attributed to time, as the only benefit was found after 8 hours of local heating.

Performance markers are just as ambiguous. Indeed, while Vaile et al. (2008) noted an increase in isometric squat strength with the use of HWI at 38° C for 15 minutes, and Kim et al. (2019) observed increased fatigue resistance with the use of 90 minutes of local heating with circulating water at 54-55° C [12,64,84], no other studies showed any performance recovery benefits in response to resistance exercise. Further, blood markers and how they are affected by heat therapy remain uncertain. From a muscle damage perspective, studies have shown that CK can be decreased when utilizing HWI [83], but may not be affected by short-wave diathermy [84]. Further, no change in Mb and LDH, as an index of muscle damage, have been observed with heat [12,83].
One of the biggest proposed benefits of heat therapy lies in its ability to affect the inflammatory response. Given that increase in vasodilation could translate to an increase in neutrophil and macrophage proliferation and infiltration, it is possible that heat can increase the pro-inflammatory response by accelerating macrophage processes. Yet, the data remains unclear. CCL2 mRNA is decreased following 90 min of local heating, although there was no reported change in the number of macrophages when compared to the sham treatment [41]. Cytokine values are shown to be affected by heat, but only pre-exercise. For instance, Vardiman et al. (2015) observed a decrease in IL-6 and TNFα with the use of 40 min of short wave diathermy pre-exercise, while HWI [83] and 100W short wave diathermy post exercise [11] showed no difference. Indeed, the benefits of heat therapy may be more prominent when used to “prime” the muscle, rather than use it to facilitate healing. Of course, given the low number of studies that have been conducted, it is hard to draw conclusions on whether heat (local or HWI) at various times may significantly impact the recovery process.
### Table 5. Heat therapy interventions for recovery from resistance-exercise induced muscle damage

<table>
<thead>
<tr>
<th>Study</th>
<th>Resistance training protocol</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al., 2019</td>
<td>20 x 15 ECC knee extensions at 30°/s</td>
<td>11 resistance trained males and females</td>
<td>90 min of local heating with water 54-55° C immediately post-exercise and 4 days post-exercise</td>
<td>↑ Fatigue resistance, VEGF mRNA ↔ ISOK peak torque at 180°/s, Soreness, Macrophage content, CAF ↓ ANGPT1 mRNA, CCL2 mRNA</td>
</tr>
<tr>
<td>Vardiman et al., 2015</td>
<td>7 x 10 2-4-s ECC leg extensions at 120% 1RM</td>
<td>15 active males</td>
<td>40 min short-wave diathermy pre-exercise</td>
<td>↔ 1RM, CK, HSP70 ↓ IL-6, TNFα</td>
</tr>
<tr>
<td>Petrofsky et al., 2017</td>
<td>3 x 5 min of 3-s ECC BW squats</td>
<td>60 healthy participants</td>
<td>8 hours of local heating immediately or 24h post-exercise</td>
<td>↔ Strength ↓ Soreness, Knee Flexion Pain</td>
</tr>
<tr>
<td>Vaile et al., 2008</td>
<td>5 x 10 eccentric leg press at 120% 1RM + 2 x 10 at 100% 1RM</td>
<td>38 resistance trained males</td>
<td>14 min of passive recovery + HWI in 38° C, CWI in 15° C, or 1 min intervals between CWI and HWI for CWT immediately post-exercise</td>
<td>↑ Isometric squat strength ↔ Peak Power, Mid-thigh girth, Mb, IL-6, LDH, Soreness ↓ CK</td>
</tr>
<tr>
<td>Castellani et al., 2016</td>
<td>24 maximal ECC actions of the elbow flexors</td>
<td>8 untrained males and females</td>
<td>100 w short wave diathermy for 15 min pre-exercise</td>
<td>↔ IL-1β, IL-10, IL-6, Mb, CK, MVC</td>
</tr>
</tbody>
</table>

ECC = eccentric, 1RM = 1 repetition maximum, BW = bodyweight, HWI = hot water immersion, CWI = cold water immersion, CWT = contrast water therapy, ISOK =
isokinetic, ANGPT1 = angiopoietin 1, VEGF = vascular endothelial growth factors, CCL2 = C-C motif chemokine ligand 2, CAF = capillaries around fiber, CK = creatine kinase, HSP70 = heat-shock protein 70, IL-6 = Interleukin-6, TNFα = tumor necrosis factor alpha, Mb = myoglobin, LDH = lactate dehydrogenase, ROM = range of motion, MVC = maximal voluntary contraction

Conclusions

Athletes and practitioners continue to look for active and passive modalities to enhance recovery via amelioration of REIMD. Currently, the body of literature continues to grow for nutritional, supplemental, cold, and hot modalities. The evidence for these modalities suggests that their effects on the recovery process are mixed, with even more sparsity on whether acute and chronic use leads to long-term benefits. Further, it is unclear if the findings from these experiments translate to untrained individuals, recreationally trained subjects, or competitive athletes. Specifically, more research is needed to further explore timing strategies of curcumin, tart cherry juice, and especially CBD. As previous work demonstrates mixed results when a loading strategy is utilized, in the future it is warranted to investigate whether prophylactic use is more appropriate than acute use immediately or soon after (24-72hr) resistance exercise. Finally, specific dosing protocols may elicit differing results. As well, a paucity in thermal strategies and their effect on REIMD is present, which is further complicated when observed with differing temperatures, localized treatment, and timing protocols. Concerns about safety and potential decrements in long-term adaptations for the modalities in this review must also be answered before definitive strategies can be developed on how and when to utilize them. Still, athletes and practitioners should work together to choose applications specific to the individuals’ goals and conditions. Finally, future studies should be directed
towards defining which strategy is most effective in timing of use in relation to exercise (pre-, intra-, post-, or a combination), the type of exercise, and potentially combining of modalities.
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Chapter III: Experimental Study

This chapter presents a complete manuscript that describes the study in traditional journal article form including an abstract, introduction, methods, results, discussion, and references. The manuscript, entitled “The effects of whole-body water immersion after exercise-induced muscle damage on exercise performance and inflammatory markers” will be submitted to the European Journal of Sports Science. It is authored by Rogelio Realzola, Jessi King, Jonathan Specht, Desmond Millender, Michael Deyhle, Christine Mermier, Flavio de Castro Magalhaes, Yu-Yu Hsiao, and Fabiano Amorim. The manuscript follows the formatting and style guidelines of the journal and the references cited are provided at the end of the manuscript.
The effects of whole-body hot water immersion after exercise-induced muscle damage on exercise performance and inflammatory markers

Running header: The effect of HWI after resistance exercise

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Abstract

This study examined the effects of hot water immersion (HWI) on exercise performance and inflammatory markers after a resistance exercise-induced muscle damage protocol. Strength trained males (n = 5) and females (n = 5) completed two experimental trials separated by at least 2 weeks in a randomized crossover design; one trial involved HWI, the other temperate water immersion (TWI). For each trial, subjects performed a muscle damaging protocol followed by HWI or TWI for up to 50 minutes. The protocol consisted of 10 sets of 8 repetitions at 70% of 1-repetition maximum with 4s eccentric, 1s concentric lifting tempo, followed by 4 sets of 20 plyometric lunges. Vertical jump, isometric and isokinetic maximal voluntary contractions, and blood markers of muscle damage and inflammation were measured prior to, and at 24h-, 48h-, and 72h-post exercise. No decreases in jump height, and isometric maximal voluntary contraction (MVC) were observed for HWI and TWI. For isokinetic MVC at 180°/s, HWI showed no decreases at any time point, while TWI decreased at every time point compared to baseline. HWI and TWI isokinetic MVC at 60°/s decreased at all time points. Soreness was increased at every time-point after HWI and TWI when compared to baseline. For blood markers, compared to baseline, creatine kinase (CK) increased at 24h-post-exercise, but was not different from baseline at 48h- and 72h-post exercise after HWI. In TWI, CK was increased at 24h-, and 72h-post exercise when compared to baseline. Myoglobin was only increased after HWI at 24h-post exercise. For inflammatory markers, C-reactive protein was increased at 24h-post exercise for HWI, but not TWI. Tumor necrosis factor-α was increased 48h- and 72h-post exercise when compared to baseline for HWI but was not increased at any time point for TWI. On the other hand, Interleukin-6 was increased
24h- and 48h-post exercise for TWI but was not increased at any time point for HWI. No changes in Interleukin-10 were observed. These findings indicate that HWI is marginally more effective than TWI to hasten exercise performance and reduce creatine kinase levels. Conversely, we speculate that inflammation lasted longer in the HWI.
Introduction

Exercise-induced muscle damage (EIMD) is typically experienced following prolonged and/or high-intensity exercise, collision sports, eccentric muscle loading, and unaccustomed exercises (Sabapathy et al., 2021; Stožer et al., 2020; Tidball & Villalta, 2010). Arguably, the primary factor for inducing EIMD is high volumes of eccentric contractions, especially when done with high force and high strain (Deyhle, Gier, et al., 2016; Hyldahl & Hubal, 2014; Stožer et al., 2020). At the cellular and sub-cellular level, EIMD is marked by damage to myofibrils, z-line streaming, and/or membrane damage, disrupted signaling between the T-tubules and sarcoplasmic reticulum, disrupted cytoskeletal organization, and changes in substrate utilization (Hyldahl & Hubal, 2014; Stožer et al., 2020). EIMD can be measured in various ways, including changes in muscle morphology, increases in specific muscle proteins detected in the plasma [creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin (Mb)] as well as increases in muscle soreness, swelling, and decreased muscle strength and range of motion (Hyldahl & Hubal, 2014; Stožer et al., 2020).

At the functional level, EIMD can limit strength and power exercise performance, and practical recommendations to prevent it have been proposed (Kellmann et al., 2018). It has been suggested that an eccentric exposure-adaptation phase with very light intensity can limit EIMD (LaStayo et al., 2014). For example, gradual loading eccentric exercise 2-3 times per week for 6-12 weeks has shown to decrease markers of EIMD (lower soreness and decreases in range of motion, reduced decrements in performance) when compared to traditional resistance training (Hyldahl & Hubal, 2014; LaStayo et al., 2014; Stožer et al., 2020). This previous exposure to eccentric exercise, known as the repeated-bout effect...
(RBE), can significantly decrease EIMD. Although the repeated bout effect is beneficial for the reduction of EIMD, the schedule of specific tournaments or sporting events throughout the season may limit the necessary exposure time to decrease EIMD. Further, training in events with naturally high eccentric demands such as sprinting, jumping, and weightlifting may still increase EIMD, given that a certain degree of fatigue in functional over-reaching is necessary for performance enhancement (Kellmann et al., 2018). Thus, as recovery and fatigue exist on a continuum, different recovery modalities are necessary for the mitigation of EIMD and its negative effects.

Local heat therapy modalities such as shortwave diathermy, water-circulating garments, heat wraps, and whirlpool therapy have been studied in the context of EIMD (Castellani et al., 2016a; K. Kim et al., 2019a; Petrofsky et al., 2017; Vardiman et al., 2015a). These methods allow for local heating with minimal or no changes in body core temperature (K. Kim, Monroe, et al., 2020). On the other hand, hot-water immersion (HWI) is a heat therapy method that can be performed with either total body immersion (below the neck) or partial water immersion (immersed up to the naval). Previous research using heat treatment, both locally and with water immersion, has shown a reduced decrement in quadriceps strength, relative force, and jump height following an eccentric exercise protocol (K. Kim, Monroe, et al., 2020; K. Kim, Reid, et al., 2020; Petrofsky et al., 2017; Vaile et al., 2008a). Similarly, heating treatments have shown improvements in soreness compared to control or temperate temperature modalities (K. Kim, Monroe, et al., 2020; Petrofsky et al., 2017; Vardiman et al., 2015b). The potential protective effects of heat are thought to be due to increased myocellular calcium efflux and enhanced ATP turnover, as well as heat-induced hyperemia (K. Kim et al., 2019a). Importantly, previous research
suggests that a dose-response relationship between the rise in core temperature and changes in inflammatory markers exists (Hoekstra et al., 2018). Another potential benefit to HWI is an increase in core temperature, which may potentially acutely increase plasma interleukin-6 (IL-6) concentration (Chaillou et al., 2022; Y. Kim & Park, 2016; Mansfield et al., 2021). Previous research has shown that HWI increases IL-6 immediately and 60 hours post-water immersion (Mansfield et al., 2021), although the effects of HWI after an eccentric exercise protocol on IL-6 have not been studied. IL-6 may act in some cases as a pro-inflammatory cytokine and is also known to act as an anti-inflammatory in the context of exercise, making conclusions on whether it has a protective or detrimental effect on EIMD difficult. It is also challenging to ascertain whether fluctuations in serum cytokines are due to systemic factors, local tissue, or both working synergistically, further confounding their potential effects. Ultimately, a change in hyperemia may have a positive effect on EIMD due to potential changes in associated increases in cytokine and myokine responses (Chaillou et al., 2022; K. Kim et al., 2019a). Importantly, hyperemia could increase delivery and extravasation of immune cells and may reduce soreness via decreased nociceceptor hypersensitization, and increased cytokine response (Mizumura & Taguchi, 2016). Still, whether IL-6 and other cytokines, such as Interleukin-10 (IL-10) and tumor necrotic factor alpha (TNFα), are increased or decreased at differing time points due to hyperemia remain to be elucidated.

Although recent developments describing the effects of HWI on modulation of inflammatory processes and exercise recovery have occurred, there remains a question of whether HWI may be beneficial in accelerating recovery from EIMD. Thus, the primary aim of the study is to examine whether HWI after an eccentric-based muscle damaging
resistance exercise protocol has positive effects on performance markers [vertical jump and maximal voluntary contraction (MVC)], markers of muscle damage (CK and Mb), ratings of perceived soreness, and markers of inflammation [C-reactive protein (CRP), IL-6, IL-10, and TNFα] over time and when a return to baseline is observed. We hypothesized that HWI will reduce decrements in performance markers, decrease soreness, attenuate blood markers of muscle damage, and decrease inflammatory markers at every time point after a muscle damaging eccentric-based resistance exercise protocol.

Methods

Subjects and ethical care

11 healthy, resistance-trained males (n = 5) and females (n = 5) participated in the study. All subjects self-reported through a Health History Questionnaire and the Physical Activity Readiness Questionnaire (PARQ) that they were participating in > 150 weekly minutes of moderate-to-vigorous exercise, which included a blend of aerobic and resistance training. Subjects reported that they were free of cardiovascular, metabolic, viral, kidney, and liver disease with no orthopedic injuries that would prevent them from exercise. Furthermore, subjects had engaged in at least two-days/week of total-body resistance exercise for > 12 months, which would qualify them as resistance-trained. All subjects reported that they were familiar with the resistance exercise performed in this study and they have had previous experience with eccentric-based training. Before exercise began, height (cm) and body mass (kg) were measured without shoes using a Holtain Limited Stadiometer (Crvmvch Dyfe, Great Britain) to the nearest 1cm, and
Detecto Digital Scale (Webb City, MOS) to the nearest 0.1 kg, respectively. In addition, body fat percentage (BF%) was calculated using a 3-site skin-fold: triceps, suprailiac and thigh for women and chest, abdomen and thigh for men (Jackson et al., 1978; Jackson et al., 1980). These values were used to estimate body density and calculate BF% (Siri, 1956).

The study was approved by the University of New Mexico Institutional Review Board, and informed consent was signed by the subjects.

**Experimental Approach**

Utilizing a balanced, randomized experimental design, participants were assigned to HWI or temperate water immersion (TWI) experimental session upon completion of a muscle damaging exercise protocol. Following a 2-week washout, the opposite water temperature was employed after performing the same exercise protocol. Participants completed an initial screening, 1-repetition maximum (1RM), familiarization of exercise testing, and baseline exercise testing prior to the muscle damaging protocol (MDP). Venous blood [for the assessment of CK activity, Mb, CRP, IL-6, IL10, and TNFα] and muscle soreness were collected prior to eccentric exercise, as well as 24-, 48-, and 72-h post-exercise. Muscle function was assessed by performing a vertical jump and MVC tests. Exercise testing occurred 24-, 48-, and 72-h post-exercise. Figure 1 provides a schematic representation of the study design.
Figure 1. Schematic detailing flow of the study. EIMD = exercise-induced muscle damage, 1RM = 1 repetition maximum, ECC = eccentric, CONC = concentric

Estimated 1RM Determination and Familiarization
The 1RM was determined for the Cybex 45° angle leg press (Brunswick Corporation, Rosemont IL, USA). Before performing each exercise, proper lifting technique and cadence were demonstrated by a researcher who is certified by the National Strength and Conditioning Association, American College of Sports Medicine, or National Athletic Training Association. After observing proper form and descriptions of each exercise, subjects performed 5-10 minutes of a self-selected warm-up before executing the first set of resistance exercise with ~50% of their self-estimated 1RM for 8-10 repetitions. Following a 3-5 min rest period, successive sets were performed, and researchers continued to add weight for each set until a successful estimated 1RM was determined, as recommended by the NSCA (Earle, 2006). All 1RM’s were determined within four attempts, within the same day. Once completed, participants were familiarized with soreness measurements, exercise testing, and water immersion. For familiarization, participants were shown the visual analog scale and were asked to mark their perceived soreness. Immediately after, participants were led to the Biodex System 4 Pro™ (Biodex, Shirley, New York, USA) and practiced both isometric and isokinetic MVCs. Next, participants were led to the Vertec vertical jump station (JumpUSA, Sunnyvale, CA, USA), to practice countermovement jumps. Finally, participants were shown the water immersion tub and practiced explaining their thermal comfort and thermal sensation scores.

**Muscle Damaging Resistance Exercise Protocol**

Upon arrival to the laboratory, pre-exercise blood samples, soreness (using a visual analog scale), and core body temperature via rectal thermistor (Thermistor 400 Series
Temperature Probe 20/Ca, Smiths Medical ASD, Inc, Minneapolis, MN, USA) and thermometer (4600 Precision Thermometer, Measurement Specialties, Shrewsbury, MA, USA) were measured. Participants then completed a 10-min self-selected dynamic warm-up followed by the muscle-damaging exercise protocol. Participants completed eccentric-based leg press (4-s lowering phase and 1-s upward phase) for 10 sets of 8 repetitions at 70% 1RM to induce muscle damage using a Cybex 45° leg press machine. Three minutes of rest were permitted between sets. Following the 10th leg press set, participants completed 4 sets of 20 consecutive plyometric lunges. Two minutes of rest were permitted between sets. The muscle-damaging protocol was adapted from a previous study conducted in our laboratory (Van Dusseldorp et al., 2020). Following completion of the muscle damaging protocol, participants were randomly selected to undergo either TWI or HWI.

**Water Immersion**

Participants were randomly selected to first sit in a tub filled with either HWI (42° C) or TWI (32° C). Participants were submerged up to their xiphoid process with their arms, head, and shoulders out of the water. The water temperature was regulated with the use of a fully submersible, portable electric immersion KD water heater (Gyeonggi-do, Republic of Korea) and a PULACO 5W mini submersible water pump (Guangzhou, China). Water temperature was checked every 5 minutes using a thermometer. In the HWI, participants were instructed to stay in the water for as long as they could, with a time cap of 50 minutes. For participants randomized into the heat trial first, time spent in TWI was based on how long participants lasted in HWI. Participants randomized into TWI first spent 50
minutes in the tub and were instructed to last as long as they could in HWI. Participants were removed from the hot water if their core temperature reached a cut off of 40.0 °C or they chose to remove themselves. Core temperature, heart rate, thermal sensation, and thermal comfort were recorded every 5 minutes.

**Visual Analog Soreness Scale**

A paper-version visual analog scale was used to assess perceived soreness. Zero centimeters represented no soreness, while 10 cm represented extreme soreness (Delgado et al., 2018). Participants were instructed to sit in a chair and indicate their soreness. Participants rated perceived soreness at pre-, 24-, 48-, and 72-h post-exercise.

**Maximum Voluntary Contractions**

Maximal voluntary isometric contractions at 90° (ISOM) and maximal voluntary isokinetic contractions at 60°/s and 180°/s (ISOK60 and ISOK180, respectively) were used to measure peak torque (N/m) on the dynamometer. Subjects were instructed to sit in the chair, were strapped in, and the arm of the device was placed on the ankle of their dominant leg. The anatomical axis of rotation was aligned to the dynamometer axis using visual inspection and manual palpation. For maximal voluntary isometric contractions, subjects completed three 5-second contractions set at an angle of 90°. Each repetition was separated by 15 seconds of rest. Once completed, subjects stayed seated for approximately 2 minutes as they were prepared for maximal voluntary isokinetic contractions. For maximal voluntary isokinetic contractions, subjects completed two sets of 3 repetitions separated by 30 seconds of rest. The first set was 3 reps at ISOK60, while
the second set was at ISOK180, both set to concentric/concentric (CON/CON)
contractions (Deyhle, Sorensen, et al., 2016).

**Vertical Jump**

Standing maximal countermovement vertical jump was determined by taking the highest
value of the three attempts using a Vertec Vertical Jump.

**Blood analysis**

All blood samples were placed into vacutainer serum separator tubes (BD, Phoenix, AZ)
and centrifuged at 22°C for 15 minutes at 2,220 g (Allegra X-14R Centrifuge, Beckman
Coulter, Brea, CA). Serum was then separated into Eppendorf vials, immediately frozen,
and stored at -80°C. For CK, samples were sent to a commercial laboratory
(QuestDirect™, Albuquerque, NM, USA) for analysis. Commercial kits were used to
perform enzyme-linked immunosorbent assays (ELISA) on Mb and CRP according to the
manufacturer instructions: myoglobin ELISA (DRG International, GmbH, Germany) and
High Sensitivity CRP ELISA (Crystal Chem, Elk Grove, IL, USA). Commercial
multiplex kits were used to perform ELISA on IL-6, IL-10, and TNFα: MILLIPLEX
Human Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel (Millipore
Sigma, Darmstadt, Germany) using a MAGPIX multiplexing platform (Luminex xMAP
Technology, San Diego, CA). Intra-assay coefficients of variation (CV) for myoglobin
and CRP were 6.9% and 3.7%, respectively. IL-6, IL-10, and TNFα intra-assay CV were
12.3%, 15.9%, and 17.8%, respectively.

**Statistical Analyses**
Dependent variables were examined for normality by Shapiro-Wilk tests, QQ plots, and histogram distribution. Assumptions of sphericity were tested using Mauchly’s test of sphericity, and violations were corrected using the Greenhouse-Geiser correction factor. All variables were normally distributed. Independent one-tailed t-tests were used to detect differences between sexes for the following demographic and descriptive variables: height, weight, BF%, age, and 1-RM. Two-way repeated measures ANOVA (time: Baseline, 24-, 48-, and 72-hours x condition: TWI and HWI) were used to detect differences in body core temperature, thermal comfort, thermal sensation, and heart rate. If a main or interaction effect was observed, Bonferroni’s multiple comparisons test was used to determine where differences between treatment and time occurred. Pairwise t-tests were used to analyze differences in ratings of perceived exertion (RPE) between exercise sessions and time spent in water immersion.

Performance and inflammatory variables

Dependent variables were examined for normality by Shapiro-Wilk tests, QQ plots, and histogram distribution. Assumptions of sphericity were tested using Mauchly’s test of sphericity, and violations were corrected using the Greenhouse-Geiser correction factor. Jump height and ISOK180 were normally distributed, while soreness, CK, Mb, CRP, IL-6, IL-10, TNFα, ISOK60, and isometric peak torque at 90° (ISOM)) were categorized as having a non-normal distribution. For all normally distributed dependent variables, one-way ANOVA was used to determine a significant main effect for time (baseline versus 24-, 48-, and 72-post exercise) to determine the number of days it took for values to return to baseline. When a main effect was observed, one-tailed pairwise t-
tests were used for post hoc analysis to detect differences between baseline and time points (24h-, 48h-, and 72h-post-exercise). To analyze non-normally distributed variables, nonparametric Friedman tests were conducted to determine significant main effects, and Wilcoxon Signed Rank Tests were used for post hoc analyses to determine differences between baseline and time points (24-, 48-, and 72- hours). Wilcoxon Signed Rank Test was used to analyze differences in CRP between baseline and 24h-post exercise.

Finally, effect sizes (ES) were calculated for all variables by the difference of delta group means, and using pooled standard deviations to arrive at Cohen’s D. For effect size, the magnitude of effect was considered small if below 0.2, medium if between 0.2 and 0.5, and large if above 0.8 (Cohen, 1988). A detailed summary of statistical data for effect sizes is presented in table 4.

GraphPad Prism 8 (GraphPad, San Diego, CA) and R version 4.2.1 (R Core Team, 2014) were used to analyze the data and generate graphs, respectively. Statistical significance was set at $p < 0.05$. All $F$ values reported are for the main or interaction effect (condition x time).

**Results**

**Demographics and Baseline Performance**

All anthropometric and baseline performance data are displayed in Table 1. Height and body mass were higher in males compared to females. 1-RM, vertical jump,
isometric peak torque at 90° were also higher in males compared to females. No significant differences between sexes were present for age, BF%, ISOK60, and ISOK180.
Table 6. Demographic, anthropometric, and baseline performance values for group (n = 10), males (n = 5), and females (n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>25.9 ± 4.8</td>
<td>27.4 ± 5.2</td>
<td>25.4 ± 4.7</td>
<td>0.76</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.8 ± 8.3</td>
<td>178.1 ± 5.5</td>
<td>166.4 ± 7.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>75.2 ± 13.8</td>
<td>84.2 ± 11.3</td>
<td>64.4 ± 7.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BF%</td>
<td>18.4 ± 4.9</td>
<td>20.4 ± 4.1</td>
<td>16.4 ± 5.1</td>
<td>0.20</td>
</tr>
<tr>
<td>1RM (kg)</td>
<td>229.3 ± 60.2</td>
<td>259.6 ± 68.7</td>
<td>192.8 ± 35.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Vertical Jump (cm)</td>
<td>45.7 ± 11.5</td>
<td>52.7 ± 9.7</td>
<td>37.3 ± 7.5</td>
<td>0.01</td>
</tr>
<tr>
<td>ISOM Peak Torque (N)</td>
<td>218.5 ± 59.8</td>
<td>250.6 ± 70.4</td>
<td>179.9 ± 24.4</td>
<td>0.04</td>
</tr>
<tr>
<td>ISOK60 Peak Torque (N/M)</td>
<td>170.5 ± 36.0</td>
<td>187.2 ± 37.9</td>
<td>150.3 ± 27.3</td>
<td>0.08</td>
</tr>
<tr>
<td>ISOK180 Peak Torque (N/M)</td>
<td>128.9 ± 29.2</td>
<td>142.7 ± 25.4</td>
<td>112.3 ± 27.2</td>
<td>0.09</td>
</tr>
</tbody>
</table>

1RM = 1 repetition maximum, BF% = body fat percentage, ISOM = maximal voluntary isometric contraction at 90°, ISOK60 = maximal voluntary isokinetic contraction at 60°/s, ISOK180 = maximal voluntary isokinetic contraction at 180°/s.

Performance Variables

Values for performance measurements (jump height, ISOM, ISOK60, and ISOK180) at baseline, 24h-, 48h-, and 72h-post-exercise are presented in Table 2. For jump height, no main effect for HWI \([F (1,14) = 53.38, p = 0.1588]\) or TWI \([F (2,19) = 1.180, p = 0.3310]\) was observed. A one-way ANOVA indicated a main effect for ISOK180 in the TWI \([F (2,17) = 8.492, p = 0.003]\), but not for HWI condition \([F (2,18) = 1.882, p = 0.1816]\). Compared to baseline, ISOK180 was lower at 24h-, 48h-, and 72h- post-exercise in TWI \((p = 0.002, p = 0.004, p = 0.01,\) respectively). Using a Friedman test, no time effect was present for ISOM in HWI \([\chi^2 (4) = 5.880, p = 0.118]\) or TWI \([\chi^2 (4) = 2.400, p = 0.4936]\). Alternatively, a main effect was present for ISOK60 at both HWI \([\chi^2 (4) = 12.84, p = 0.005]\) and TWI \([\chi^2 (4) = 15, p = 0.002]\). For HWI, Wilcoxon signed rank test indicated ISOK60 values at 24h- \((p = 0.007)\), 48h- \((p = 0.019)\), and 72h- post-exercise \((p = 0.019)\) were lower than at baseline. Similarly, ISOK60 values for TWI were lower than baseline at 24h- \((p = 0.003)\), 48h- \((p = 0.005)\), and 72h- post-exercise \((p = 0.019)\).
Effects sizes comparing TWI and HWI for the performance measurements (jump height, ISOM, ISOK60, and ISOK180) of the delta values between baseline and 24h-, 48h-, and 72h- post-exercise are presented in table 4. For jump height, effect size analysis revealed small positive effects between baseline and 24h- and 48h- post-exercise comparing TWI and HWI. A positive medium effect was observed between baseline and 72h- post-exercise. For ISOM, effect size analyses revealed a small negative effect at 24h-post, and small positive effects between TWI and HWI at 48h- and 72h-post. On the other hand, effect sizes were small and negative for ISOK60 when comparing baseline and 24h- post-exercise, while resulted in medium positive effects at 48h- and 72h- post-exercise between TWI and HWI. Finally, for ISOK180, positive effect sizes were medium when comparing baseline and 24h- and 72h- post-exercise, while a large effect was present between baseline and 48h- post-exercise when comparing TWI and HWI (Table 4).
Table 7. Jump and MVC measurements. All data presented as means ± SD. (n = 10)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Baseline</th>
<th>24h-POST</th>
<th>48h-POST</th>
<th>72h-POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jump Height (cm)</td>
<td>HWI</td>
<td>43.7 ± 11.9</td>
<td>43.0 ± 10.4</td>
<td>42.8 ± 9.3</td>
<td>45.6 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>43.4 ± 10.2</td>
<td>41.0 ± 8.7</td>
<td>42.7 ± 9.2</td>
<td></td>
</tr>
<tr>
<td>ISOM Peak Torque (N/m)</td>
<td>HWI</td>
<td>215.2 ± 62.0</td>
<td>202.3 ± 46.8</td>
<td>214.2 ± 49.3</td>
<td>225.5 ± 45.3</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>206.8 ± 40.6</td>
<td>206.8 ± 40.6</td>
<td>198.7 ± 42.4</td>
<td></td>
</tr>
<tr>
<td>ISOM Peak Torque (N/m)</td>
<td>HWI</td>
<td>144.6 ±</td>
<td>141.1 ± 26.1*</td>
<td>26.3*</td>
<td>153.3 ± 24.8*</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>170.9 ± 38.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISOK60 Peak Torque (N/m)</td>
<td>HWI</td>
<td>141.6 ± 28.2*</td>
<td>143.7 ± 27.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>129.5 ± 30.7</td>
<td>122.3 ± 31.9</td>
<td>122.6 ± 32.5</td>
<td></td>
</tr>
<tr>
<td>ISOK180 Peak Torque (N/m)</td>
<td>HWI</td>
<td>116.9 ± 29.9</td>
<td>122.3 ± 31.9</td>
<td>122.6 ± 32.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>108.0 ± 32.9*</td>
<td>32.3*</td>
<td>113.1 ± 31.2*</td>
<td></td>
</tr>
</tbody>
</table>

ISOM = maximal voluntary isometric contraction at 90°, ISOK60 = maximal voluntary isokinetic contraction at 60°/s, ISOK180 = maximal voluntary contraction at 180°/s, HWI = hot water immersion, TWI = temperate water immersion

* signifies significantly different from baseline

Perceptual Markers

For soreness, using a Friedman test it was observed a main effect for HWI \( \chi^2(5) = 28.88, p < 0.001 \) and TWI \( \chi^2(4) = 22.58, p < 0.001 \). In the HWI condition, soreness values were high at 24h- \( p = 0.001 \), 48h- \( p = 0.001 \), and 72h- post-exercise \( p = 0.001 \) compared to baseline values. Similarly, in the TWI condition soreness values were high at 24h- \( p = 0.001 \), 48h- \( p = 0.001 \), and 72h- post-exercise \( p = 0.004 \) compared to baseline (Figure 2). When comparing the effect sizes between TWI and HWI, negative small/medium at 24h-, medium at 48h-, and small at 72h-post-exercise were observed (Table 4).
Figure 2. Changes in perceived soreness 24-, 48-, and 72-hour -post water immersion. Measurements are reported as medians ± interquartile range. TWI = temperate water immersion, HWI = hot water immersion
* Significantly different than baseline for TWI, p ≤ 0.05
# Significantly different than baseline for HWI, p ≤ 0.05

Blood Markers of muscle damage

CK and Mb were analyzed with a Friedman test. It was observed a main effect in both HWI and TWI \[\chi^2(4) = 10.68, p = 0.014, \text{ and } \chi^2(4) = 8.939, p = 0.030,\] respectively] for CK. Compared to baseline, CK was higher at 24h- post-exercise \(p = 0.019\) but not at 48h- and 72h- post-exercise \(p = 0.31\) and \(p = 0.36\), respectively) for HWI. Alternatively, compared to baseline, CK was higher at 24h- \(p = 0.013\) and 72h-post-exercise \(p = 0.042\), but not at 48h- post-exercise \(p = 0.067\) for TWI (Figure 3A). For CK, effect size analyses between TWI and HWI indicated a small negative effect between baseline and 24h-, and medium negative effects between baseline and 48h- and 72h-post-exercise.
For Mb, it was observed a main effect for HWI $[\chi^2(4) = 9.121, p = 0.028]$, but not for TWI $[\chi^2(4) = 3.240, p = 0.356]$. Mb values were higher in the HWI between baseline and 24h- post exercise ($p = 0.013$), while showing no difference between baseline and 48h- ($p = 0.246$) and 72h- post exercise ($p = 0.410$) (Figure 3B). For Mb, effect size analyses between TWI and HWI indicated a small/medium positive effect between baseline and 24hr post-exercise, and a small negative effect between baseline and 48h- and 72h- post-exercise (Table 4).

Figure 3. Change in blood markers of exercise-induced muscle damage after 24-, 48-, and 72-hour-post exercise. A. Creatine kinase (U/L). B. Myoglobin (pg/mL). All data are reported as medians ± interquartile range.
TWI = Temperate water immersion, HWI = Hot water immersion
* Significantly different than baseline for TWI, $p \leq 0.05$
# Significantly different than baseline for HWI, $p \leq 0.05$

Inflammatory Markers

Using a Wilcoxon signed rank test, it was observed higher CRP values between baseline and 24hr- post-exercise for the HWI condition ($p = 0.008$) but not for the TWI ($p = 0.116$) (Figure 4A). For CRP, effect size analyses between TWI and HWI indicated a
small negative effect between baseline and 24hr post-exercise for TWI and HWI comparisons (Table 4).

A significant main effect for IL-6 was observed for TWI ($\chi^2(4) = 11.64, p = 0.009$), but not HWI ($\chi^2(4) = 4.680, p = 0.197$). Post-hoc analyses indicated high IL-6 values at the 24h- ($p = 0.005$) and 48h- ($p = 0.005$) post-exercise compared to baseline, but not for 72h- post-exercise comparison ($p = 0.116$) for TWI (Figure 4B). No significant main effect was observed for IL-10 in the HWI ($\chi^2(4) = 2.520, p = 0.472$) or TWI ($\chi^2(4) = 1.440, p = 0.696$) conditions (Figure 4D). While no main effect was reported for TNFα in the TWI condition ($\chi^2(4) = 5.061, p = 0.1674$), it was observed in the HWI condition ($\chi^2(4) = 8.510, p = 0.037$). Post hoc analyses indicate higher TNFα values at 48h- ($p = 0.007$) and 72h- post-exercise ($p = 0.005$), but no difference at 24h-post-exercise ($p = 0.15$) (Figure 4C).

For IL-6, effect size analyses between TWI and HWI indicated a negative medium effect between baseline and 24h-, and a positive medium effect at 72h- post-exercise, while a small negative effect at 48h- post-exercise was present for TWI and HWI comparisons (Table 4). In addition, IL-10 effect sizes were small and negative between baseline and 24h- and 48h- post-exercise, while a positive medium effect was present at 72h-post-exercise when comparing TWI and HWI. Finally, TNFα effects sizes were positive and medium between baseline and 24h-, and positive and large at 48h- and 72h-post-exercise when comparing TWI and HWI (Table 4).
Figure 4. Changes in markers of inflammation after 24-, 48-, and 72-hour-post exercise. A. C-reactive protein (pg/mL). B. Interleukin-6 (pg/mL). C. TNF-α (pg/mL). D. Interleukin-10 (IL-10). Data are reported as medians ± interquartile range. TWI = Temperate water immersion, HWI = Hot water immersion

* Significantly different than baseline for TWI, p ≤ 0.05
# Significantly different than baseline for HWI, p ≤ 0.05

Physiological Variables and Subjective Feelings

Two-way repeated measures ANOVA was used to analyze differences in body core temperature, thermal comfort, thermal sensation, and heart rate at pre-muscle damaging protocol (Pre-MDP), pre-water immersion (Pre-WI), post-water immersion
(Post-WI) time points between HWI and TWI. Further, max values (Max) were also analyzed between HWI and TWI. Values are presented in Table 3. For body core temperature, a main effect for time \((F(2,38) = 53.09, p < 0.0001)\) and interaction (time x condition) \((F(3,60) = 32.79, p < 0.0001)\) were observed. Bonferroni’s multiple comparisons tests reported no differences between HWI and TWI in Pre-MDP \((p > 0.99)\) and Pre-WI \((p > 0.99)\). Body core temperature was higher at Post-WI \((p < 0.0001)\) and Max \((p = 0.001)\) in the HWI compared to TWI. Main effects for time \((F(2,35) = 66.90, p < 0.0001)\) and interaction (time x condition) \((F(2,40) = 74.69, p < 0.0001)\) were observed for thermal comfort. Thermal comfort values were higher at Pre-WI \((p < 0.001)\), Post-WI \((p < 0.001)\), and Max \((p < 0.001)\) for HWI compared to TWI. A main effect for time \((F(2,33) = 43.47, p < 0.001)\) and interaction (time x condition) \((F(2,40) = 69.66, p < 0.001)\) were observed for thermal sensation. Thermal sensation values were higher at Pre-WI \((p = 0.014)\), Post-WI \((p < 0.001)\), and Max \((p < 0.001)\) for HWI compared to TWI. Finally, main effects for time \((F(1,28) = 21.08, p < 0.001)\) and interaction (time x condition) \((F(2,40) = 42.75, p <0.001)\) were reported for heart rate. Heart rate values were higher for HWI compared to TWI at Post-WI \((p < 0.001)\) and Max \((p < 0.001)\). Pairwise t-tests revealed no significant differences \((p = 0.75)\) in ratings of perceived exertion (RPE) between HWI \((8.57 \pm 0.90 \text{ RPE})\) and TWI \((8.71 \pm 0.77 \text{ RPE})\) muscle-damaging sessions. Further, pairwise t-tests revealed a significant difference in time spent in HWI \((26 \pm 9.37 \text{ minutes})\) and TWI \((39 \pm 14.5 \text{ minutes})\) \((p = 0.02)\).
**Table 8.** Physiological variables and subjective feelings pre- and post-water immersion interventions (n = 10)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Pre-MDP</th>
<th>Pre-WI</th>
<th>Post-WI</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Core Temperature (°C)</td>
<td>HWI</td>
<td>37.45 ± 0.49</td>
<td>38.17 ± 0.26</td>
<td>38.86 ± 0.39*</td>
<td>39.15 ± 0.32*</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>37.47 ± 0.58</td>
<td>38.21 ± 0.55</td>
<td>37.40 ± 0.21</td>
<td>38.34 ± 0.53</td>
</tr>
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<td>Thermal Comfort</td>
<td>HWI</td>
<td>-</td>
<td>1 ± 1*</td>
<td>4 ±0*</td>
<td>4 ± 0*</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>-</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Thermal Sensation</td>
<td>HWI</td>
<td>-</td>
<td>6 ± 1*</td>
<td>8 ± 1*</td>
<td>9 ± 1*</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>-</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>HWI</td>
<td>-</td>
<td>107 ± 13</td>
<td>135 ± 18*</td>
<td>136 ± 17*</td>
</tr>
<tr>
<td></td>
<td>TWI</td>
<td>-</td>
<td>96 ± 16</td>
<td>75 ± 14</td>
<td>99 ± 12</td>
</tr>
</tbody>
</table>

* = significant difference compared to TWI. HWI = hot water immersion, TWI = temperate water immersion, Pre-MDP = pre-muscle damaging protocol, Pre-WI = pre-water immersion, Post-WI = post-water immersion, Max = max value
Table 9. Summary of all between-group effects for vertical jump, MVC, soreness and markers of muscle damage, and inflammatory markers. (n = 10)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Performance Variables</th>
<th>HWI vs TWI</th>
<th>ES</th>
<th>Effect Magnitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Jump Height</strong></td>
<td>Pre-24h</td>
<td>.00</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>.30</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>.47</td>
<td>Small/Medium</td>
<td></td>
</tr>
<tr>
<td><strong>ISOM</strong></td>
<td>Pre-24h</td>
<td>-.10</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>.39</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>.32</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td><strong>ISOK60</strong></td>
<td>Pre-24h</td>
<td>-.02</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>.42</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>.31</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td><strong>ISOK180</strong></td>
<td>Pre-24h</td>
<td>.51</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>.82</td>
<td>Large</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>.57</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td><strong>Soreness</strong></td>
<td>Pre-24h</td>
<td>-.49</td>
<td>Small/Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>-.67</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>-.32</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td>Pre-24h</td>
<td>-.33</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>CK</strong></td>
<td>Pre-24h</td>
<td>-.34</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>-.52</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>-.70</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td><strong>Myoglobin</strong></td>
<td>Pre-24h</td>
<td>.47</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>-.30</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>-.44</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>Pre-24h</td>
<td>-.55</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>-.29</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>.53</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td><strong>IL-10</strong></td>
<td>Pre-24h</td>
<td>-.23</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>-.10</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>.54</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td><strong>TNFα</strong></td>
<td>Pre-24h</td>
<td>.53</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-48h</td>
<td>1.05</td>
<td>Large</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre-72h</td>
<td>1.16</td>
<td>Large</td>
<td></td>
</tr>
</tbody>
</table>

ISOM = maximal voluntary isometric contraction at 90°, ISOK60 = maximal voluntary isokinetic contraction at 60°/s, ISOK180 = maximal voluntary contraction at 180°/s, CRP = C-reactive protein, CK = creatine kinase, IL-6 = Interleukin-6, IL-10 = Interleukin-10, TNFα = Tumor necrosis factor α
Discussion

The aim of the present study was to assess the effects of HWI (42°C) on muscular performance, perceived muscle soreness, markers of skeletal muscle tissue damage, and inflammation during a recovery period after an eccentric-based lower-body resistance exercise session. The main findings were that HWI reduced the decrement in ISOK180 performance from 24h- to 72h-post-exercise, while after TWI performance decreased at all time points. For blood markers, CK was increased at 24h-post exercise, but not at 48h- and 72h-post exercise for HWI, while TWI had increased CK at 24h-, and 72h-post exercise. For inflammatory markers, CRP was increased at 24h-post exercise for HWI, but not TWI. TNF-α was increased 48h- and 72h-post exercise when compared to baseline for HWI, while no increases were observed at any time point for TWI.

To the author’s knowledge, the present study is the first to independently investigate the effects of a whole-body HWI protocol on exercise performance and markers of muscle damage following a lower body, eccentric-based resistance exercise session in resistance trained males and females. Our resistance exercise MDP protocol was able to reduce ISOK60, ISOK180 (for TWI), as well as increase soreness and markers of skeletal muscle damage. Thus, we feel confident that the MDP was able to effectively induce muscle damage for up to 72 hours to effectively study recovery. These changes have been seen in previous MDP protocols similar to ours where the aim was to elicit moderate amounts of muscle damage (K. Kim et al., 2019a; Vaile et al., 2008b; Vandusseldorp et al., 2018).

Performance Variables
In the present study, ISOK60 was lower at every time point compared to baseline for both HWI and TWI. Importantly, decreases in ISOK180 were present at every time point for TWI, but not for HWI. Further, no differences were observed for jump height and ISOM. This lack in a reduction in ISOM is not congruent with previous studies that have reported decrements in isometric peak force for up to 10 days after the MDP (Castellani & Young, 2016; Vaile et al., 2008c). These differences might be related to the muscle groups used and subject population, as Castellani et al. (2016) used elbow flexors compared to quadriceps in the present study, and their subjects were untrained, leading to larger decrements in peak force. Regarding the protocol used to measure isometric strength, Vaile et al. (2008) utilized an isometric squat to measure peak force, although Castellani et al. (2016) did use three maximal contractions for 3s at 90°, which was similar to our protocol (3 maximal contractions for 5s at 90°). In the present study, ISOK180 values were not different than baseline at all time points after HWI. This lack of difference for ISOK180 with HWI at any time point is consistent with previous studies showing attenuation of peak torque 48h-post exercise (Castellani et al., 2016b) and up to 72h-post exercise (K. Kim et al., 2019b) with hot water interventions. To our knowledge, no studies have investigated the effect of heat therapy (water immersion or local heat therapy) on ISOK60. In summary, our MDP protocol was able to induce reductions in MVC with isokinetic movement but not with isometric contractions. Further, the HWI was able to aid recovery of muscle function only at low strength or more power-based contractions (ISOK180). Based on these data, it is speculated that HWI may help to maintain the ability to maintain powerful contractions, but not strength-based contractions.
Effect size was calculated for differences between HWI and TWI for all variables at all timepoints. The main findings from ES analyses suggest that HWI may have small/medium positive effects in ISOM, ISOK60, and Jump Height. Further, a large positive effect magnitude is present for ISOK180, especially at later time points. This large positive effect size for ISOK180 is aligned with the faster MVC recovery in the HWI described above. In brief, these results indicated that HWI is more effective than TWI to hasten exercise performance after a resistance based MDP.

**Soreness**

In the present study, soreness was increased at every timepoint compared to baseline for both HWI and TWI. The findings that HWI does not decrease soreness at any time point contradicts previous studies indicating that heat therapy, albeit locally, reduces pain (Petrofsky et al., 2017). Importantly, our study methodology varies from previous research. For instance, Petrofsky et al. (2017) applied local heat therapy (heat wraps) on the quadriceps for 8 hours immediately after eccentric resistance exercise and observed that soreness was significantly lower 24h- and 48h-post-exercise when compared to a control group. The authors speculated that the reduction in perceived pain in heat treatments is believed to be due to increases in tissue blood flow and the “washing away” of metabolites, allowing for quicker healing (Petrofsky et al., 2017). Although the authors indicated that heat therapy was effective to reduce soreness compared to control, it was not reported when soreness returned to baseline values. On the other hand, the present study is in agreement with Vaile et al. (2008) who found that a muscle damaging protocol increased soreness, and 14 minutes of HWI was not effective in returning soreness to baseline values at 24h-, 48h-, and 72h-post-exercise. Despite the fact the present study
used a longer (26 vs 14 minutes) and hotter water immersion protocol (42°C vs 38°C), HWI was not effective in reducing soreness after a resistance based MDP. We speculate that the lack of effect of HWI on soreness may have to do with the short amount of time participants were immersed in the hot water, as previous research showed differences sooner when participants utilized heat treatments for longer periods of time, such as 8 hours (Petrofsky et al., 2017), and 90 minutes of local heat treatment (K. Kim et al., 2019a).

Changes in perceived soreness are thought to be caused in part by heat-induced hyperemia potentially reducing nerve growth factors (K. Kim et al., 2019a), which play a role in mechanical hyperalgesia (Hyldahl & Hubal, 2014). Similarly, heat-induced hyperemia was proposed to increase the content of chemoattractant CCL2, and consequently, CD68+ macrophages, which play a key role in the inflammatory and soreness response (K. Kim et al., 2019a). However, Kim et al. (2019) found that increased protein expression of CCL2 during recovery did not lead to subsequent changes in the dynamic and magnitude of macrophage recruitment in humans. Thus, it is unclear whether heat-induced changes to soreness had to do with macrophage infiltration modifications.

Finally, ES analyses suggest that HWI may have a small/medium negative effect depending on time point, but especially 48h-post resistance exercise. The small effect size magnitude aligns with the results showing that no decreases in soreness are apparent at any time point. These results indicate that HWI may not be effective in decreasing soreness after eccentric-based resistance exercise.

**Blood markers of muscle damage**
CK and Mb both were higher at 24h-post exercise compared to baseline in HWI. For TWI, however, Mb was not different at any time point, although CK was higher at 24h- and 72h-post exercise, while trending towards being higher at 48h-post exercise ($p = 0.06$). These differences in CK values compared to baseline indicate that HWI may be more effective in decreasing CK values than TWI. These results are contrary to previous studies utilizing local short-wave diathermy which observed elevated CK up to 120h-post exercise (Castellani et al., 2016b). Although the effects of HWI on Mb remain to be elucidated, the present study demonstrates that Mb was higher at 24-hr-post exercise. These results are inconsistent with previous work. For instance, Vaile et al. (2008) utilized HWI and observed no difference in Mb 24h-post exercise. Alternatively, Mb was increased with local diathermy 72h-post exercise (Castellani et al., 2016b), and has been shown to be increased up to 48h-post exercise following an eccentric-resistance exercise protocol (Philippou et al., 2009). The small effect magnitude for Mb aligns with our results that Mb is only increased 24h-post exercise after HWI. Further, medium effect magnitude for CK corresponds with the results showing a return to baseline by 24h-post exercise. These results indicated that while HWI may not affect Mb, HWI may be effective in decreasing CK levels after eccentric-based resistance exercise.

**Inflammatory markers**

CRP was increased at 24h-post exercise compared to baseline for HWI, but not for TWI. To our knowledge, this is the first study utilizing heat after a damaging resistance exercise protocol to analyze the effects on CRP. HWI has potential to acutely increase markers of inflammation, such as CRP, and plasma IL-6 concentration (Brunt et al., 2018; Hoekstra et al., 2020). While exercise and passive heating independently increases IL-6
concentrations, we can carefully assume that it may also increase CRP, although it is unclear whether HWI might have an additive effect (Faulkner et al., 2017). It is interesting that CRP levels are increased in HWI while there is no increase in IL-6 (and vice versa in TWI), given that CRP production is stimulated by IL-6 (Del Giudice & Gangestad, 2018). Although CRP is generally thought of as a catch-all for inflammation, it exists in two isoforms that originate from differing sites and have opposite effects. The pentameric isoform of CRP, secreted from the liver, diffuses in plasma and has a range of largely anti-inflammatory effects. Indeed, it is mainly involved in resolution of inflammation, induces the anti-inflammatory M2 phenotype in macrophages, and can recruit complement inhibition factors that may function to limit indiscriminate tissue damage (Trial et al., 2016). On the other hand, monomeric CRP is highly pro-inflammatory, thus inducing the M1 macrophage response (Del Giudice & Gangestad, 2018). Importantly, given that we did not analyze levels of CRP in tissue, it is impossible to ascertain whether the CRP has a net pro- or anti-inflammatory effect. Further, CRP begins to rise within 4-6 hours after the onset of inflammation, and peaks at approximately 36 hours (Gabay & Kushner, 1999), which might explain differences being observed in this study. As we used a 24h-post exercise time point, maximal levels of CRP may have not hit peak values before 36 hours.

IL-6 levels were not increased at any time point for HWI. Alternatively, a significant increase was observed for TWI at 24h- and 48h-post exercise. IL-6 not being increased after a heating treatment in the present study is in line with previous research showing decreases in IL-6 up to 72-hours-post exercise using diathermy when compared to baseline (Vardiman et al., 2015a), and no changes using diathermy compared to baseline
(Castellani et al., 2016a). No increases in IL-6 after heat treatment might help to explain the lack of difference in anti-inflammatory cytokine responses, as IL-6 is known for stimulating IL-10 release. These results are interesting, given that a normal heat treatment with no exercise increased IL-6 compared to baseline (Mansfield et al., 2021). Although, while no exercise was performed in the study conducted by Mansfield et al. (2021), time spent in the heat was much longer (60 minutes) compared to our study (~26 minutes). It may also be possible that perturbations in cytokines may occur before 24hr-post exercise, which was not measured in our study (Hoekstra et al., 2021). The medium effect magnitude for IL-6 at earlier (24h-) and later (72h-) time points is in line with our results that show increases in IL-6 for TWI, but not HWI. Indeed, further research examining the effect of HWI on IL-6 after eccentric-based resistance exercise is needed to understand why IL-6 levels are not increased when compared to baseline. Further, no increase in IL-6 after HWI should be studied for potential long-term effects.

Arguably the most interesting effect of HWI may have been its role on TNFα. Although TNFα was not increased at any time point for TWI, it was higher at 48h- and 72h-post compared to baseline for HWI. Importantly, increased TNFα at 48h- and 72h-post exercise after HWI in the present study is different from previous heat studies (Castellani et al., 2016b). Indeed, Castellani et al. (2016) reported no changes compared to baseline in TNFα (pre-exercise to 24h-post exercise). The difference in results is interesting and may be due to the heat treatment being performed pre-exercise in the Castellani et al. (2016) study, which is known to “prime” the muscle and potentially decrease damage and the inflammatory process (Hoekstra et al., 2021; Pedersen, 2013; Vardiman et al., 2015a). It is well established that TNFα acts as a pro-inflammatory,
potentially implicating HWI to be detrimental to the recovery process, especially given that no time effect of TNFα was present in the TWI group. Still, the TNFα increase in response to exercise is markedly different than when due to severe infection (Pedersen, 2013). Lower TNFα levels may be beneficial for the recovery process at later stages of the recovery process, further confounding the effect of high TNFα at later time points. Finally, the effect magnitude being large at 48h- and 72h-post exercise presents ripe opportunity to examine the potential benefits that may occur with HWI. Although there are links between the inflammatory response, blood markers of muscle damage, and performance recovery, the present data simply introduces new information to add to the body of literature.

**Physiological and Subjective Variables in Heat**

As expected, body core temperature, both Post-WI and Max-WI, was significantly higher in HWI compared to TWI. Further, thermal comfort, thermal sensation, and heart rate were significantly higher in HWI compared to TWI. The temperature of the HWI protocol (42° C) was chosen as previous research indicates that skeletal muscles can reach > 40° C (Morris et al., 2005). There were no significant differences in Pre-MDP between HWI and TWI. Heat exposure is known to increase body temperature, heart rate, and redistribution of blood flow (Brunt et al., 2016). Not surprisingly, the present study shows similar results to previous studies that revealed significant changes in heart rate and core temperature in HWI compared to TWI (Brunt et al., 2016; Mansfield et al., 2021; Viitasalo et al., 1995).

Perceptual responses (thermal comfort and thermal sensation) in the present study were higher (7.8 ± 0.4 vs 8.7 ± 0.67 for thermal sensation, and 3.0 ± 1.0 vs 3.90 ± 0.30
for thermal comfort) when compared to a previous study that used hot water immersion at 42° C for 60 minutes, although no pre-WI exercise was utilized (Mansfield et al., 2021). The difference in these perceptual responses might be related to the starting body core temperature, as subjects in the present study had undergone an eccentric resistance-exercise protocol immediately prior to HWI, which raised body core temperature by approximately 1° C. It is worth noting that in the present study, thermal comfort and thermal sensation were significantly different Pre-WI (1±1 for HWI, 0 ± 0 for TWI). Although there is no definitive reason why, the similarity in body core temperature Pre-WI between both sessions leads us to speculate that the temperature in the room where water immersion was applied was higher in the HWI compared to TWI. Although room temperature was not measured, it is speculated that anticipation of getting into the hot water, as well as water evaporation from the heating system changed the room temperature and increased the thermal responses of the subjects.

**Limitations**

We acknowledge that there are limitations in the current study. Our exercise protocol, which used eccentric-based lower body resistance exercise, may not be directly translatable to “real world” scenarios, such as those that occur with athletes, recreational athletes, and people working in high stress/strain occupations. Although moderate changes in MVC, jump, and soreness were present, it may be beneficial for future studies to incorporate different exercise modes to the EIMD protocols, such as upper body eccentric resistance exercise bouts, sprint training, high intensity interval work, or intermittent exercise sessions to better mimic “real world” scenarios. In addition, although we allowed participants to voluntarily end heat trials, it is difficult to ascertain
whether potential changes were due to increases in magnitude of core temperature, or due
to duration. Future studies should investigate whether warm, but longer durations in HWI
would have different effects than shorter, but hotter HWI sessions. Finally, the
participants in the present study were resistance trained individuals. One would be
cautious to extrapolate the data in the current study to be used for untrained individuals,
or athletes in a specific sport.

CONCLUSION

In summary, HWI following a resistance eccentric-based exercise sessions showed
positive effects on ISOK180 returning to baseline values compared to TWI. Further, HWI
following resistance eccentric-based exercise sessions returned CK to baseline values
quicker than TWI, but not for Mb. Finally, some markers of inflammation (CRP and
TNFα) were elevated longer in HWI, but not for TWI. CRP was increased in HWI at
24h-post exercise, and TNFα was increased at 48h- and 72h-post exercise. Conversely,
IL-6 was increased in TWI at 48h- and 72h-post exercise, with no change in HWI. No
increases in IL-10 were observed at any time point. Longitudinal studies, studies within
different populations, and studies comparing the time of treatment vs the magnitude of
heat are needed to ascertain whether the current protocols would lead to disparate
outcomes.
Chapter III References


Chapter IV: Summary, Conclusion and Recommendations

Summary

As discussed in Chapter 1, exercise induced muscle damage (EIMD) is typically experienced following training that one is unaccustomed to. Although a myriad of recovery strategies have been used to ameliorate EIMD, each seems to have a cost to benefit ratio. Specifically, cold therapy has potential acute benefits (decreases in soreness) that must be weighed with potential chronic detriments (reduced hypertrophy). As such, the logical next step is to study whether the use of heat benefits recovery after EIMD both acutely and longitudinally. While the use of heat has shown some promising results for recovery, studies vary from local heat treatment (short-wave diathermy) to full body hot water immersion (HWI). Further, no studies have investigated the effect of HWI on markers of inflammation (such as C-reactive protein (CRP), Inteleukin-6 (IL-6), Interleukin 10 (IL-10), and tumor necrosis factor alpha (TNFα)). Therefore, it was hypothesized that HWI would decrease blood markers of muscle damage (creatine kinase (CK) and myoglobin (Mb)), decrease ratings of perceived soreness, improve decrements in performance markers (jump height and maximum voluntary isometric and isokinetic contractions), and markers of inflammation (CRP, IL-6, IL-10, TNFα) sooner after muscle damaging exercise compared to temperate water immersion (TWI).

In Chapter 2, the literature review outlined an overview of EIMD, as well as the current literature on the use of several supplements (curcumin, tart cherry juice, and CBD) and thermal (cold and heat) recovery protocols after a bout of resistance exercise-induced muscle damage (REIMD). Although the literature on recovery strategies is large,
the data analyzing recovery processes specifically after resistance exercise is lacking. Given that REIMD is affected differently than damage incurred with endurance exercise, intermittent exercise, or other eccentric protocols such as downhill running and jumping, it is logical to speculate that certain recovery modalities may or may not be beneficial for increased recovery in REIMD. More research is necessary on the proposed recovery strategies, but it is possible that appropriate modality, dosage, and timing of recovery strategies can have a profound impact on REIMD.

As portrayed in Chapter 3, the experimental study described in the current manuscript involved a repeated-measures design in which 10 recreationally trained lifters performed a session of muscle damaging resistance training followed by water immersion in temperate or hot water. The muscle damaging protocol consisted of 10 sets of 8 eccentric repetitions (4-second lowering phase and 1-second upwards phase) at 70% 1RM on a plate-loaded 45° leg press as well as 4 sets of 20 consecutive plyometric bodyweight lunges. The water immersion protocol consisted of either HWI at 42° C or (TWI) at 32° C for up to 50 minutes. Soreness, jump height, isometric and isokinetic maximal voluntary contraction, markers of muscle damage (CK and Mb), and markers of inflammation (CRP, IL-6, IL-10, TNF-α) were measured pre-exercise, as well as 24h-, 48h-, and 72h-post exercise. Data revealed that HWI attenuated decrements in maximal voluntary isometric contraction at 180°/s (ISOK) at all time points, while TWI did not. Similarly, CK returned to baseline by 48h-post exercise in HWI, while TWI remained elevated at 72h-post exercise. While TNF-α was higher at later time points (48h- and 72h-post exercise) in HWI, in TWI it was not different in any time point. Importantly, TWI saw increased levels of IL-6, which was not present in HWI. Finally, HWI and TWI
seems to have no effect on jump height, isometric maximal voluntary contraction (ISOM), IL-10, and soreness. These results suggest that HWI may only be slightly beneficial for positively impacting EIMD compared to TWI, but it may induce a greater early inflammatory response that might be related to faster recovery and greater long-term adaptation.

**Conclusion**

When HWI is used after a bout of eccentric resistance exercise, there are positive effects on isokinetic maximal voluntary contractions at 180°/s returning to baseline before TWI. Further, CK returned to baseline values quicker than TWI, but Mb did not. Finally, some markers of inflammation (CRP and TNFα) were elevated longer in HWI but were not for TWI, while IL-6 was increased only after TWI. Importantly, no increases in IL-10 were observed. Although speculative, it is possible that hyperthermia either at the body core or at the skeletal muscle tissue that occurs with HWI may be beneficial for recovery. More research is needed to corroborate these findings, but it seems that HWI has the potential to affect performance markers and inflammatory markers based on the magnitudes of effects.

**Recommendations**

Due to the modest, but positive effects on recovery after REIMD, the use of heat post muscle damaging exercise deserves additional study. Future researchers should replicate our design and provide data for acute molecular signaling within skeletal muscle samples to determine if HWI and TWI lead to different upregulation of protein involved in the skeletal muscle hypertrophy pathway (i.e., mTORC1), ATP turnover, or
adaptations to resistance exercise. From there, longitudinal studies are necessary to see if HWI and TWI have divergent outcomes for muscular strength and hypertrophy. To isolate the effect of whether the length of time spent in HWI or a greater temperature is necessary for acute and longitudinal adaptations, future studies on HWI and its effects on EIMD should utilize different temperatures and time spent immersed in water.