Investigating the effects of hot water immersion on neuromuscular function in human dorsiflexor muscles

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ABSTRACT

Hot water immersion (HWI) is a popular post-exercise recovery intervention, however its effects on neuromuscular function are inconsistent, potentially due to differences in immersion duration. This study investigated time and intramuscular temperature-dependent changes in neuromuscular function throughout 1h of 44°C HWI. It was hypothesized that acute HWI (<30 min) would not affect neuromuscular function, whereas prolonged HWI (>30 min) would improve muscle contractility by substantially elevating intramuscular temperature. Fourteen participants (8M:6F) partook in two separate visits consisting of 1) 1h HWI at 44°C without exercise (HWI-only), and 2) non-fatiguing exercise followed by 1h post-exercise HWI at 44°C (Ex + HWI). Isotonic peak power increased after 10 min in the HWI-only (111±11.8%, p<0.05), and Ex + HWI (115.7±18.0%, p<0.05) conditions, whereas maximal isometric strength remained unchanged. Increases in contractile measures indicated an intramuscular mechanism, confirmed by re-cooling showing the effect was temperature-dependent, supporting HWI-induced muscle heating as a strategy to enhance peak power.

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The need to understand does not require a justification beyond itself, it is curiosity that carries a person to discovery. What follows these acknowledgements are not merely a list of names but a small confession. There is no mind that works alone, every insight is borrowed light, refracted through the minds of others before reaching a page. I thank everyone who lent me their trust, belief, and experiences. This work carries your fingerprints even where your names do not appear.

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LIST OF ABBREVIATIONS

HWI	Hot-water immersion
CWI	Cold-water immersion
MVC	Maximal voluntary contraction
RTD	Rate of torque development
HRT	Half relaxation time
Ca^{2+}	Calcium
O_2	Oxygen
ATP	Adenosine triphosphate
PAP	Post-activation potentiation
SERCA	Sarcoplasmic reticulum calcium ATPase
$[Ca^{2+}]_i$	Intracellular calcium
HSP	Heat shock proteins
SR	Sarcoplasmic reticulum

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction on the effects of hot water immersion

1.1.1 Overview of hot water immersion as a post-exercise intervention

The search for interventions that enhance athletic performance and accelerate the recovery of neuromuscular function has been a central focus in sports science. Among the many types of recovery and performance-enhancement modalities employed (i.e., cold water immersion, sauna, cryotherapy), hot water immersion (HWI) has emerged as a promising intervention due to its ability to non-invasively increase intramuscular temperature (Chaillou et al., 2022; Rodrigues et al., 2025; Denny et al., 2025). Typically defined as full or partial body immersion in water temperatures ≥38.5°C, HWI represents a form of passive heating that, unlike active warm-up procedures, can rapidly elevate tissue temperature without inducing neuromuscular fatigue (Menzies et al., 2025).

Among traditional recovery interventions, cold-water immersion (CWI) has been most commonly used by athletes and coaches, operating on the presumption that reduced intramuscular temperature minimizes inflammation and perceived muscle soreness (Chaillou et al., 2022; Choo et al., 2022). However, recent evidence suggests that the effects of CWI are ineffective or even detrimental to muscle function (Malekzadeh et al., 2026). Muscle function encompasses both strength and power. For athletes, strength and power are complementary but distinct determinants of athletic performance: strength refers to the maximal force that a muscle or muscle group can produce, whereas power reflects the rate at which force can be generated (i.e., power = force x velocity). Recent findings indicate that CWI impairs muscle function with the most pronounced impairments in peak power occurring within 10 min post-immersion (Malekzadeh et al., 2026). Unlike CWI which decreases muscle temperature with cooling, the use of HWI increases muscle temperature with heating and is presumed to improve blood flow, oxygen (O_2) delivery to the

tissue, alleviate perceptions of fatigue, and enhance muscle function (Heinonen et al., 2011; Chaillou et al., 2022; Gagnon et al. 2025). Despite these proposed benefits, only a limited number of studies have linked the changes in muscle contractile function (i.e., improved rate of torque development, and half relaxation time) to increases in intramuscular temperature with heating (Sargeant, 1987; Rodrigues et al., 2025; Davies & Young, 1983; Ranatunga & Wylie, 1983). Moreover, evidence for HWI as a post-exercise recovery intervention remains inconsistent, with some studies suggesting benefits (Rodrigues et al., 2021; De Ruiter et al., 1999; Cheng et al., 2017; Clarke et al., 1963), while others demonstrating no improvements in the recovery of muscle function (i.e., maximal strength and power output) (Vaile et al., 2008; Méline et al., 2021; Solsona et al., 2023; Newlands et al., 2024). One potential explanation for these inconsistencies in the application of post-exercise HWI may be due to differences in the temperature and duration of HWI employed, which will influence intramuscular temperature differently and its effects on muscle contractile function. A lower water temperature 38.5-42°C applied for a shorter duration (<30 min) may result in negligible effects on muscle function, as reported in several studies (Jackman et al., 2023; Solsona et al., 2023; Gustafsson et al., 2025; Newlands et al., 2024). In contrast, studies employing higher temperatures (43-46°C) for prolonged exposures (>30 min) have demonstrated more favorable outcomes for the recovery of muscle function (Clarke et al., 1963; Rodrigues et al., 2023; Rodrigues et al., 2025). Second, the type of preceding exercise task (e.g., endurance, resistance, high intensity) may determine HWI responsiveness, as the induction of fatigue is different with each exercise task and its recovery may not be responsive to HWI (Bishop, 2003; Vaile et al., 2008; Gustafsson et al., 2025). While its use as a post- exercise intervention has become common in sport practices, evidence regarding its effectiveness has been

inconsistent and contingent on achieving sufficient intramuscular temperature elevation through water temperature and immersion duration.

This review will investigate the optimal prescription of HWI (i.e., temperature and duration) required to see benefits on neuromuscular function. Specifically, this review will synthesize findings from both isolated muscle preparations and *in vivo* human studies to distinguish temperature-dependent peripheral mechanisms (e.g., altered cross-bridge kinetics, calcium handling) from potential central mechanisms. In addition, this review will consider changes in muscle function associated with elevated intramuscular temperature, while acknowledging that underlying mechanisms require further investigation. This review will differentiate between acute (≤ 30 min) and prolonged (≥ 30 min) HWI protocols, hypothesizing that its effects are expected to be most pronounced during the prolonged phase where tissue temperature and blood flow remain elevated.

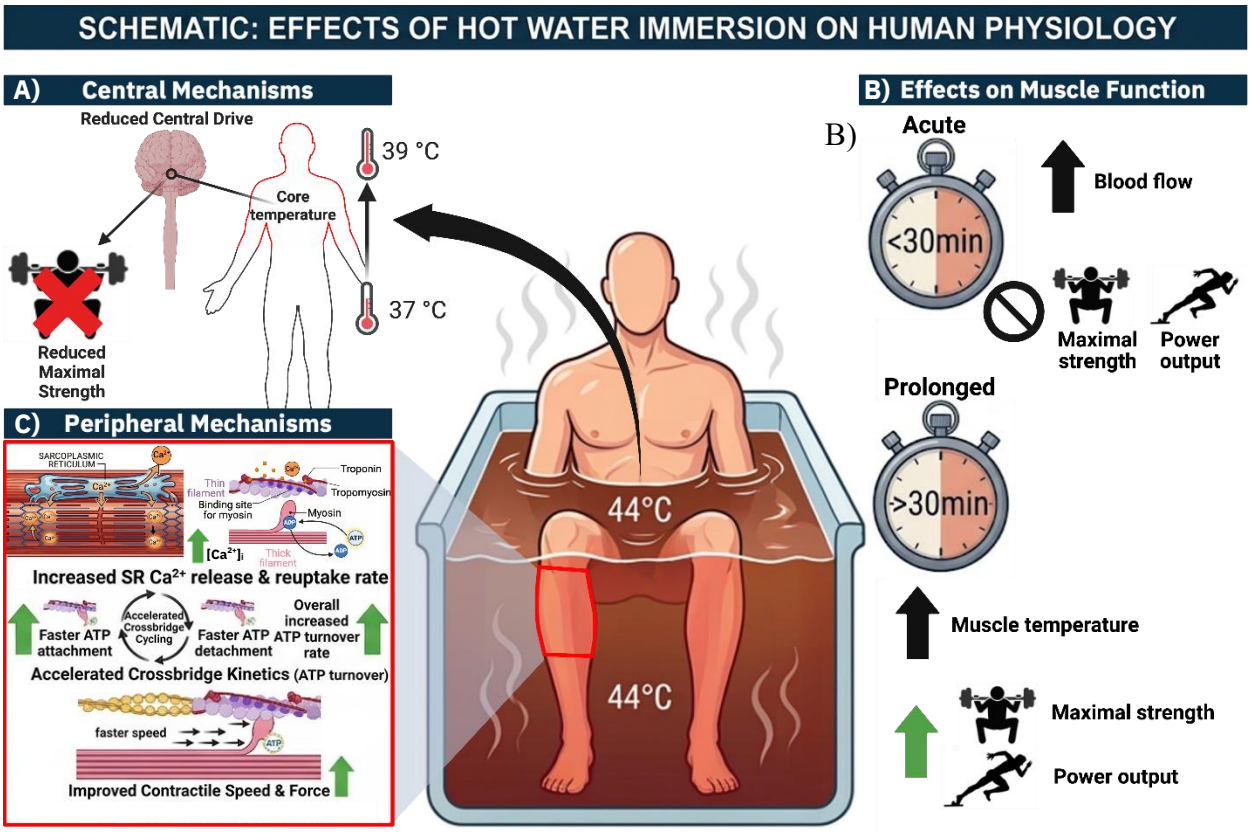


Figure 1: The physiological effects of hot water immersion on neuromuscular function

A) Systemic immersion in hot water induces an acute increase in core body temperature, which influences neural drive. Consequently, this impaired central drive results in a marked decrease in voluntary muscle activation and maximal voluntary strength. B) Local immersion in hot water immersion yields improvements blood flow, and contractile speeds but the effects on the recovery of muscle function are dependent on intramuscular temperature and immersion duration. The effects of temperature on muscle function following acute (<30 min) exposure are not equivalent to prolonged exposure (>30 min). Prolonged immersion substantially elevates local muscle temperature to a greater extent, allowing for a greater response. This adaptation is believed to be primarily due to muscle mechanics and not improvements to blood flow. C) At the cellular level, these muscular improvements in speed and force are potentially mediated by improved crossbridge cycling kinetics and intracellular calcium ($[Ca^{2+}]_i$) dynamics within the muscle cell. Elevated muscle temperature accelerates calcium (Ca^{2+}) release kinetics, and potentially improving its affinity to troponin C.

1.2 Physiological basis of temperature and neuromuscular function

1.2.1 Effects of intramuscular temperature on muscle contractile function

A rise in intramuscular temperature can influence skeletal muscle physiology, with some evidence linking this alteration to improvements in contractile properties of skeletal muscle (Close & Hoh, 1968; Lännergren & Westerblad, 1987; De Ruiter et al., 1999; Davies & Young, 1983; Sargeant, 1987; Ranatunga & Wylie, 1983). Early work by Asmussen and Boje (1945) was the first to demonstrate that passively increasing muscle temperature to 38°C enhanced contraction speed in knee extensor and elbow flexor muscles, establishing that muscle temperature modulates contractile velocity and that higher temperatures (i.e., 39°C) facilitated faster chemical processes within the muscle. Building on this observation, Davies and Young (1983), reported that an increase in intramuscular temperature from 36.8°C to 39.7°C was sufficient to meaningfully improve time to peak tension and half-relaxation time (HRT) in human triceps surae. Consistent with this, Sargeant (1987) added to these findings, demonstrating that heating the human quadriceps muscle to a temperature of 39.3°C from 36.6°C improved peak knee extensor force. Contrastingly De Ruiter et al. (1999) using electrically stimulated tetanic contractions of the adductor pollicis, reported that maximal isometric force remained unchanged at 25°C to 37°C but found an improvement in the RTD at 37°C. The findings of the previous studies reveal that elevated muscle temperature influences force production and contractile kinetics, with improvements observed when muscle is warmed above normal physiological temperature, though the specific temperature-force relationship varies depending on method of activation, and the temperature range examined.

Only a limited number of studies employing HWI have observed modest improvements in muscle strength (Rodrigues et al., 2021; Clarke et al., 1963), typically involving either prolonged

immersion durations (>30 min) or higher water temperatures (>45°C). Most studies report negligible effects on maximal strength (Binkhorst et al., 1977; De Ruiter & De Haan, 1999; Jackman et al., 2023). For example, Binkhorst et al. (1977) observed no change in maximal isometric handgrip strength following 30 min of HWI at 39°C. Similarly, De Ruiter and De Haan (1999) reported that passive heating of the quadriceps muscles improved RTD and HRT during both electrically evoked and voluntary contractions but did not alter maximal tetanic strength. Collectively, these findings suggest that increases in muscle temperature do not have meaningful effects on maximal isometric torque production, whereas velocity and power may be more susceptible to temperature alterations. One potential explanation for why a rise in intramuscular temperature may not have an effect on maximal strength, as seen in previous literature, is that a muscle operates near its absolute torque-generating capacity during a maximal isometric contraction. Thus, an acute rise in intramuscular temperature is unlikely to provide further improvements in maximal strength (i.e., ceiling effect) (De Ruiter & De Haan, 1999; Binkhorst et al., 1977). In contrast to the negligible effects observed in maximal strength, elevated muscle temperature appears to influence submaximal neuromuscular function. A recent study by Rodrigues et al. (2023) demonstrated that a 2.4°C rise in muscle temperature reduced the motor unit discharge rate required to maintain a submaximal contraction at 20% MVC. These findings suggest that while an elevated muscle temperature may not raise the 'ceiling' of maximal strength, it lowers the 'cost' of submaximal force production by enhancing peripheral responsiveness to neural commands.

The underlying mechanisms for HWI-induced improvements in muscle contractile kinetics likely involve enhanced peripheral processes, including accelerated Ca^{2+} transients and increased myofilament sensitivity, which collectively improve the speed of torque production (Allen et al.,

2008; Ranatunga, 2010; Rodrigues et al., 2023). As a result, the same torque can be achieved with reduced neural drive, highlighting that these temperature dependent improvements are intrinsic to the muscle rather than neural (Rodrigues et al., 2023).

In conclusion, an elevated intramuscular temperature appears beneficial for muscle function, particularly for skeletal muscle contractile kinetics (RTD, and HRT), while its effects on maximal isometric strength appear modest or negligible (Allen et al., 2008; Ranatunga, 2010; Rodrigues et al., 2023). This discrepancy is often attributed to a potential "ceiling effect" during maximal efforts and differences in activation mode (voluntary vs. involuntary). However, it is important to note that while many studies examine outcomes immediately after passive heating, no study shows evidence of how the duration of improvements in contractile kinetics versus muscle strength and power evolve over time. This is particularly important because the duration of HWI effects muscle temperature, which directly influences the contractile kinetics. Whether the benefits to RTD and HRT persist longer than any potential changes in maximal force or power output remains unclear, and whether these temporal profiles differ between voluntary and involuntary contractions.

1.2.2 Full body versus partial limb immersion: Effects on central and peripheral muscle function

Elevated muscle temperature with HWI has been shown to yield some beneficial effects on neuromuscular function. However, the outcome of these effects depends on extent of body immersion during HWI protocols. Previous studies demonstrate considerable variation in immersion approaches, ranging from localized limb heating to full-body immersion, and the evidence from these studies show markedly different and sometimes opposing responses on neuromuscular function. Neuromuscular function represents the ability of the nervous system to activate skeletal muscle and generate force or power, involving both a central component (brain and spinal cord) that initiate neural drive to the peripheral components (skeletal muscle) which execute the contractile response. One reason for the differences in neuromuscular function observed in prior studies is due to the differential manipulation of core versus local temperature.

Full body immersion introduces systemic responses such as elevated core temperature, increased heart rate, cardiac output, and cardiovascular strain (Rowell, 1974). When applied post-exercise in the heat, this additional core thermal load can prolong or exacerbate hyperthermia and central fatigue, reducing neural drive to the muscle even when muscle temperature is favourable (Morrison et al., 2004; Thomas et al., 2006; Nybo & Nielsen, 2001). While the rise in heart rate and cardiac output have negligible effects on the intrinsic properties of skeletal muscle, a modest rise in core temperature (37°C to 39°C), prompts the central nervous system to reduce voluntary activation (VA) to the muscle, thus reducing maximum voluntary contraction torque (MVC) (Nybo & Nielsen, 2001; Todd et al., 2005; Morrison et al., 2004; Thomas et al., 2006; Rodrigues et al., 2023). In contrast, partial limb immersion selectively manipulates muscle temperature while maintaining core temperature within basal physiological ranges. This approach allows researchers to isolate the peripheral effects of elevated muscle temperature on muscle function, including

improved contractile kinetics, enhanced rate of torque development, and faster relaxation times, without the systemic influence of heating on elevated core temperature, leading to impaired voluntary activation (Thomas et al., 2006; Rodrigues et al., 2023). Work by Thomas et al. (2006) supports this evidence by demonstrating that voluntary activation and MVC torque of the plantar flexors are impaired during passive heating primarily by elevated core temperature, rather than changes in muscle temperature (Brazaitis & Skurvydas, 2010; Thomas et al., 2006; Todd et al., 2005). This indicates that the peripheral benefits of warming, such as the increased rate of torque development observed by De Ruiter et al. (1999), can be effectively masked if the protocol induces systemic effects seen in full body immersion protocols.

Supporting this central and peripheral distinction, Rodrigues et al. (2023) demonstrated that a 2.4°C rise in intramuscular temperature of the vastus lateralis muscle induced by 42°C HWI reduces the motor unit discharge rate required to achieve a given submaximal torque (Rodrigues et al., 2023). While no change in maximal isometric strength was observed following a return in core temperature, improvements in contractile kinetics (i.e., RTD) and reduced motor unit discharge rates persisted with an elevated muscle temperature (Rodrigues et al., 2023). Unlike full body heating which inhibits central drive and MVC torque, this finding suggests that when core temperature is controlled, but the muscle is heated, the nervous system shows improved neural input and preserved maximal strength. Moreover, the distinction between local vs. core temperature becomes increasingly important when assessing the effects of passive heating and muscle function *in vivo*. While its effects on muscle temperature are enhanced contraction speed, and motor unit efficiency, a concomitant rise in skin temperature can also result in improvements in muscle function. Skin temperature improves muscle function primarily through enhanced

cutaneous reflex responses that improve sensorimotor feedback without affecting central drive (Howe et al., 2023).

Collectively, these findings from previous studies have demonstrated that the effects of temperature on neuromuscular function are driven by both systemic (core temperature), and peripheral factors (intramuscular temperature). While a rise in muscle temperature via partial limb immersion appears to improve muscle function, a rise in core temperature following full body immersion has been shown to reduce voluntary activation of the muscle and subsequently decrease MVC torque. This indicates that the associated benefits of elevated muscle temperature are primarily caused by peripheral factors rather than central (neural) contributions. Importantly, skin temperature elevation can further influence function through enhanced cutaneous reflexes and sensorimotor feedback, independent of core or muscle temperature. Thus, optimizing heating protocols requires careful consideration of core, skin, and muscle temperature factors.

1.3 Mechanisms driving temperature-dependent improvements

1.3.1 Cellular and molecular adaptations

An increase in muscle temperature elicits intramuscular alterations, with foundational work by Hill (1938) demonstrating that muscle contractile performance increases with higher temperatures. Hill was first to show that a 10°C increase in the temperature of isolated frog sartorius muscle can improve its shortening velocity during electrically stimulated tetanic contractions. While the physiological mechanisms underpinning these effects have not been fully elucidated (Ranatunga et al. 2010), subsequent studies have supported and extended previous observations suggesting multiple temperature-dependent processes contributing to improved skeletal muscle function.

These improvements include accelerated cross-bridge attachment and detachment kinetics (Ranatunga et al. 2010), increased rates of adenosine triphosphate (ATP) turnover (Edwards et al., 1975) and enhanced myosin ATPase activity (Bárány, 1967), which facilitate faster cross-bridge cycling. The increased enzymatic activity at higher temperatures facilitates a more rapid hydrolysis of ATP, providing the chemical energy for faster cross-bridge cycling (Bárány, 1967). This is supported by Edwards et al. (1975), who documented increased rates of ATP turnover in heated muscle, confirming that the metabolic machinery supplying the contractile apparatus becomes more efficient with elevated temperature. In addition to crossbridge mechanics, evidence also suggests that muscle heating can improve Ca^{2+} handling by augmenting sarcoplasmic reticulum (SR) Ca^{2+} release and reuptake via faster sarcoplasmic reticulum calcium ATPase (SERCA) pump activity (Watanabe et al, 2018; Power et al., 2016). In line with this interpretation, Cheng et al., 2017 demonstrated that in both human muscle and isolated mouse fibers, recovery of submaximal force, intracellular tetanic $[Ca^{2+}]_i$, and fatigue resistance following exhaustive exercise was

accelerated by heating (36–38°C) relative to cooling (Cheng et al., 2017). The release of Ca^{2+} into the myoplasm results in the binding of Ca^{2+} to troponin C to initiate cross bride formation and subsequently producing force development. Ca^{2+} reuptake to the SR because of it unbinding from troponin C leads to muscle relaxation. As previously mentioned, observations by De Ruiter and De Haan (1999) reported that passive heating improved the rate of force development and muscle relaxation during both electrically evoked and voluntary contractions but did not alter maximal tetanic strength. While an increase in intramuscular temperature can improve skeletal muscle contractile properties (i.e., RTD and HRT). A proposed but not yet fully established explanation is that this reflects peripheral mechanisms such as improved Ca^{2+} handling and myofilament sensitivity. However, direct causal evidence for this specific link is still needed to fully elucidate the mechanisms. Studies on skinned muscle fibers demonstrate a leftward shift in the force-Ca^{2+} relationship at higher temperatures, indicating that more force is produced at a given Ca^{2+} concentration (Stephenson & Williams, 1985; Richards et al., 2025). This is mechanistically explained by temperature-dependent increases in the affinity of troponin C for Ca^{2+} and altered kinetics of the regulatory proteins (Kobayashi et al., 2005). However, it remains unclear if passive heating would increase Ca^{2+} sensitivity as the interaction between Ca^{2+} and troponin C is an exothermic reaction, suggesting that the binding affinity should decrease with higher temperatures (Potter et al. 1977).

Beyond these acute contractile improvements, moderate elevations in muscle temperature (~40°C) also trigger adaptive cellular responses through heat shock protein (HSP) activation. HSPs respond to thermal and cellular stress, functioning to protect proteins, and maintain cellular proteostasis (Moseley, 1997). However, there seems to be an upper threshold by which heat stimulates protein degradation more than protein synthesis (Baracos et al. 1984; Luo et al. 2000).

Specifically, *in vitro* models have demonstrated that when muscle temperatures exceed 42°C, muscle protein degradation begins (Baracos et al. 1984; Essig et al. 1985). Thus, it seems that there is a potential ceiling by which increasing muscle temperature is beneficial for skeletal muscle function. Therefore, manipulating muscle temperature up to a maximum of 41 °C represents a safe and effective strategy for heating skeletal muscle (Faulkner et al., 2013).

In addition to these effects, an elevated muscle temperature may enhance post-activation potentiation (PAP), as warmer muscles exhibit faster contractile kinetics, this may be a potential mechanism explaining these observed effects (Blazevich & Babault, 2019). PAP refers to a transient enhancement of muscle force and power following a conditioning contraction, typically mediated by phosphorylation of myosin regulatory light chains and increased Ca^{2+} sensitivity of the contractile apparatus (Blazevich & Babault, 2019; Sweeney et al., 1993). A warmer muscle typically exhibits faster contractile kinetics and enhanced Ca^{2+} sensitivity, conditions that are known to favor the expression of PAP. Passively elevated intramuscular temperature may amplify PAP responses by accelerating cross-bridge kinetics and increasing the responsiveness of the contractile apparatus to Ca^{2+}, thereby enhancing rapid force production and power output during subsequent high-intensity efforts (Blazevich & Babault, 2019; Sargeant, 1987, Grange et al., 1993; Ranatunga, 2010).

In summary, a rise in muscle temperature within optimal physiological range (34 to 41°C) can serve as a beneficial modulator for improved skeletal muscle function as evidenced by the molecular responses in HSPs. However, temperatures exceeding this range (i.e., >42°C) may result in protein degradation. These improvements are driven by changes in myosin ATPase activity, ATP turnover, and potentially Ca^{2+} sensitivity, acting locally within the muscle fiber to enhance skeletal muscle function independently of central neural drive. These molecular adaptations with

muscle heating explain the improved force production, and enhanced contractile kinetics (i.e., RTD, HRT) that occur during exercise and contribute improved muscle function (Rodrigues et al., 2022; Power et al., 2016; Cheng et al., 2017; Denny et al., 2025).

1.3.2 Metabolic responses: blood flow and oxygen delivery

Beyond isolated muscle preparations, intramuscular temperature also interacts with local vascular responses including blood flow, muscle O_2 delivery and extraction which are presumed benefits with an elevated muscle temperature. These responses are presumed to aid in removal of waste products especially following exercise where metabolite concentrations are elevated (Cady et al., 1989).

Local increases in muscle temperature have been shown to substantially elevate muscle blood flow. Johnson and Kellogg (2010) observed that local heating via water immersion increases cutaneous and muscle blood flow, with peak hyperemia occurring at water temperatures between 40–42°C. These responses are driven by both endothelial-dependent (nitric oxide-mediated) and endothelial-independent pathways, which significantly increase local perfusion (Minson et al., 2001). Heinonen et al., (2011) showed that an increase in intramuscular temperature by ~4°C increased calf muscle perfusion in humans. Traditionally this increase in blood flow has been thought to support skeletal muscle function through metabolic means (i.e., increased local perfusion, enhancing muscle O_2 delivery and extraction at the tissue) (Chaillou et al., 2022). However, observations by Gagnon et al. (2025) demonstrate that while local heating improves blood flow delivery of the quadriceps muscle, it does not translate to greater O_2 delivery or utilization during exercise. Cheuvront and Kenefick (2014) further propose that in tasks where oxidative capacity is not a limiting factor, increases in blood flow do not yield improvements in

muscle function. This indicates that HWI-induced hyperemia is likely a secondary physiological response, and the primary benefits to contractile function are driven by non-metabolic, temperature-dependent mechanisms (i.e., faster cross-bridge kinetics). Consistent with this, passive heating induced improvements in cross-bridge formation (Edman and Andersson, 1968) and muscle shortening velocity (Edman and Hwang, 1977) appear to be independent of muscle blood flow. While the correlation between heat and improved kinetics is clear, the exact physiological mechanisms by which blood flow and temperature interact to drive these processes remain to be fully established.

These findings suggest that the effects of HWI on hyperemia is likely a secondary response to outcomes driving improved muscle function. The primary functional benefits are temperature-dependent (crossbridge kinetics) rather than from enhanced O_2 delivery and/or extraction at the muscle. However, further discussion is warranted on what the precise mechanistic contribution of blood flow and O_2 delivery are on skeletal muscle contractile function.

1.4 Practical application and protocol effectiveness

1.4.1 Time and temperature influence the effectiveness of hot water immersion

While it is well established that an elevated muscle temperature can enhance skeletal muscle contractile properties (De Ruiter & De Haan, 1999), the effectiveness of HWI in improving neuromuscular function can be determined by the immersion temperature employed as well as the immersion duration (Rodrigues et al., 2025), and its effects on intramuscular temperature. However, an important limitation that exists in the current literature is the inconsistency in reporting intramuscular temperature, the immersion temperature or immersion duration. While some studies provide intramuscular temperature measurements without reporting immersion

temperatures or durations (De Ruiter & De Haan, 1999; Rodrigues et al., 2021), others report only immersion duration and water temperature without reporting intramuscular temperature (Binkhorst et al., 1977; Jackman et al., 2023; Newlands et al., 2024). This discrepancy makes it difficult to establish a definitive timeline for improvements in neuromuscular function as it relates to intramuscular temperature changes.

The importance of intramuscular temperature for muscle function is further emphasized when considering the detrimental time dependent effects of cooling. Malekzadeh et al. (2026) linked a decrease in peak power following cold water immersion (CWI) directly to impaired contractile kinetics (i.e., RTD and HRT) at just 10 min, but not maximal voluntary isometric strength, which impaired later (30 min). While an elevated muscle temperature has shown promise in improving the contractile kinetics, it appears that temperature does not uniformly affect all contractile properties of muscle. Furthermore, whether these temperature-dependent improvements relate to an increase in strength and power is not fully elucidated.

In addition, while a water temperature of >38.5°C typically defines HWI protocols (Menzies et al., 2025), previous studies commonly employ protocols ranging from 39-46°C, with durations spanning 10 to 120 minutes (Rodrigues et al., 2025; Chaillou et al., 2022). These inconsistencies in temperature and duration can greatly influence the effects HWI has on intramuscular temperature and its effects on skeletal muscle function. Studies examining the acute (<30 min) effects of HWI on muscle function have been inconsistent due to variation in immersion temperature whereas studies employing higher temperatures (43-46°C) or prolonged exposures (>30 min) have more consistently shown positive effects of HWI on muscle function. For instance, a study by Jackman et al. (2023) reported that 10 min of HWI at 40°C did not change maximal

strength. In another study measuring power, Solsona et al. (2023) demonstrated that 20 min of HWI at 41°C did not increase post-exercise power output. Consistent with this, Newlands et al. (2024) found that 25 min of HWI at 38.5-40°C did not enhance power following exercise. In contrast to lower temperatures (i.e., 38.5-41°C) and acute durations (i.e., <30 min), HWI protocols using higher temperatures (e.g. 42-46°C) or longer durations (>30 min) have demonstrated favorable changes in muscle function. Rodrigues et al. (2021) reported that immersion at 42 °C for 90 min increased muscle strength in human quadriceps muscles. Moreover, De Ruiter et al. (1999) demonstrated that elevating muscle temperature in a 45°C bath for 20 min accelerated the RTD and HRT but did not translate into greater tetanic strength in human hand muscles. In addition to this, Clarke et al., (1963) demonstrated that 10 mins of HWI at 46°C increased the acute recovery of handgrip strength following exercise.

Finally, it is also important to consider the timing between immersion and task and how this may influence performance outcomes. Performance benefits appear greatest immediately post-immersion where intramuscular temperature would be highest. This is further supported by recent findings by Rodrigues et al., 2025, demonstrating that 45 min of HWI at 42°C of the waist down can acutely enhance countermovement-jump height and peak force compared to a thermoneutral control 34°C. However, these performance improvements were short lived and return to pre-immersion values within 15 minutes. Although muscle temperature was not directly measured, the loss of performance benefit may be explained by the return of intramuscular temperature toward basal values as previous studies have indicated that intramuscular temperature typically declines back to basal temperature values within 1 h post- immersion (Fuchs et al., 2020; Faulkner et al., 2013; Dablainville et al., 2025). It is important to consider that most of these studies demonstrating a return in basal muscle temperature involve an acute HWI protocol (<30 min). Another potential

factor influencing the effectiveness of HWI may be muscle size, as studies have shown that larger muscle groups such as the quadriceps femoris muscle often require longer durations (i.e., 90 to 120 min) to elicit significant strength changes (Rodrigues et al., 2021). In contrast, smaller muscles, such as those in human hand muscles (De Ruiter et al., 1999; Clarke et al., 1963) and plantar flexors (Thomas et al., 2006) often report rapid changes in contractile kinetics.

To summarize, these findings suggest that the effectiveness of HWI in enhancing muscle function primarily depends on the water immersion temperature, and duration. While HWI protocols generally employ immersion temperatures above 38.5°C, widely varying temperature (39-46°C) and duration (10 to 120 min) combinations create mixed intramuscular temperature responses, which likely underlie the discrepancies behind the use of HWI and its effects on muscle strength and power. Short duration (<30 min) and low temperature protocols (38.5-41°C) often fail to elicit substantial effects on muscle function, whereas higher temperatures (45-46°C) or longer durations (>30 min) are more likely to improve muscle function (Rodrigues et al., 2025). While higher HWI temperatures seem ideal in rapidly elevating intramuscular temperature, these high temperatures often provoke substantial discomfort in humans, which can limit immersion duration and obscure potential time-dependent effects on performance (Fuchs et al., 2020). Finally, it is important to note that improvements in performance following HWI are most pronounced immediately post immersion and likely dissipate as intramuscular temperature returns toward baseline, typically within 1 h.

1.5 Overall conclusion

The use of HWI on neuromuscular function have been relatively under explored compared with CWI (Chaillou et al., 2022). However, the effectiveness of post-exercise HWI on

neuromuscular function remains inconsistent (Vaile et al., 2008; Méline et al., 2021; Rodrigues et al., 2021). When investigating the effects of HWI on neuromuscular function following exercise, negligible effects are often reported (≤ 30 min), whereas modest improvements are seen in the later stages (≥ 30 min) (Jackman et al., 2023; Clarke et al., 1963; Rodrigues et al., 2025). This review investigated the effects of elevated muscle temperature on skeletal muscle function, building on foundational work evaluating its beneficial physiological effects (Hill, 1938; Close & Hoh, 1968). Evidence indicated that HWI can modulate skeletal muscle contractile performance (i.e., RTD and HRT), yet less is known of what these effects may look like *in vivo* (Davies & Young, 1983; De Ruiter & De Haan, 1999). While an improvement in the RTD and HRT are prevalent with heating, whether that may respond to equal improvements in maximal strength and peak power is unclear in previous literature (Binkhorst et al., 1977; Rodrigues et al., 2023). More importantly, the precise time- and intramuscular temperature dependent effects by which these outcome measures may be favourable are not fully quantified (Chaillou et al., 2022; Menzies et al., 2025). Beyond functional outcomes, the temperature dependent mechanisms of HWI include accelerated cross-bridge cycling, improved Ca^{2+} handling, increased myofilament sensitivity, and augmented muscle blood flow (Ranatunga, 2010; Debold, 2006; Watanabe et al., 2018; Heinonen et al., 2011). It is important to note that the evidence suggests that the beneficial effects with HWI are primarily driven by peripheral factors rather than central (neural) contributions (Thomas et al., 2006; Rodrigues et al., 2023).

Rationale

Elevating intramuscular temperature above basal physiological levels enhances skeletal muscle contractile performance, particularly improving the RTD and HRT. These effects are largely attributed to temperature-dependent increases in cross-bridge cycling rate, faster SR Ca^{2+}

handling, and greater myofilament Ca^{2+} sensitivity, which collectively improve the speed of force generation. However, the extent by which temperature-induced improvements in contractile performance translate to meaningful improvements in *in vivo* performance remains inconsistent across HWI studies. One potential reason is that the prescription of HWI varies widely in both temperature and duration, resulting in differences in intramuscular temperature and its effects on muscle function. Moreover, the time-dependent effects of neuromuscular function following prolonged (>30 min) HWI has not been fully elucidated, thereby limiting evidence-based recommendations for using HWI as either a recovery or performance intervention. While skeletal muscle performance is improved with elevated muscle temperature, evidence shows that increases in core temperature can impair muscle function. Therefore, selectively isolating the contributions of intramuscular temperature with local heating, is critical for understanding why some HWI studies may report improved muscle function while others do not. Passive heating has been alluded to enhancing cross-bridge kinetics, SR Ca^{2+} handling, and potentially PAP. Thus, a higher initial muscle temperature may increase peak power. This study will assess the time- and temperature dependent changes in neuromuscular function during prolonged 1 h of 44°C HWI.

Hypothesis

It was hypothesized that 1) acute (< 30 min) HWI at 44°C will not affect muscle function due to limited increase in intramuscular temperature, 2) prolonged (> 30 min) HWI at 44°C will enhance skeletal muscle function through gradual increases in intramuscular temperature, and 3) re-cooling the muscle back to basal physiological temperature will remove any temperature-dependent improvements in muscle function.

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CHAPTER 2: MANUSCRIPT

2.1 Introduction

Thermal therapies such as cold water immersion (CWI) are commonly used post-exercise interventions aimed at enhancing the recovery of muscle function. Although CWI remains a widely adopted thermal therapy recovery intervention, accumulating evidence suggests that its effects on skeletal muscle contractile function are either negligible or potentially detrimental (Chaillou et al., 2022; Choo et al., 2022; Malekzadeh et al., 2026; Richards et al., 2025). In contrast, hot water immersion (HWI) is another form of thermal therapy that has received comparatively less attention despite its potential benefits on post-exercise recovery and muscle function (Périard et al., 2017; Chaillou et al., 2022; Rodrigues et al., 2022).

Hot water immersion (HWI) is a post-exercise intervention that has been implemented among athletes due to its proposed role in accelerating post-exercise recovery (Vaile et al., 2008; Méline et al., 2021; Chaillou et al., 2022). However, there is some debate as to whether the recovery of muscle function (e.g., maximal strength and power) is improved by HWI, with some studies suggesting benefits (Clarke et al., 1963; De Ruiter et al., 1999; Rodrigues et al., 2021), while others highlighting no effects with its use (Rodrigues et al., 2023; Jackman et al., 2023; Gustafsson et al., 2025). Discrepancies in the effectiveness of HWI in previous studies likely stem from variability in the temperature and duration of HWI employed, which consequently effects intramuscular temperature and thus muscle contractile function (De Ruiter et al.,1999; Rodrigues et al., 2025). HWI protocols are typically defined by water temperatures exceeding 38.5°C (Chaillou et al., 2022; Rodrigues et al., 2025, Menzies et al., 2025), yet considerable variability exists across studies, with temperatures ranging from 39-46°C, and durations spanning 10 to 45 minutes (Clarke et al., 1963; Chaillou et al., 2022; Rodrigues et al., 2025). These inconsistencies

in temperature and duration can greatly influence intramuscular temperature and its effects on skeletal muscle function. Studies examining the acute (< 30 min) effects of HWI have generally reported negligible improvements in muscle function (Jackman et al., 2023; Solsona et al., 2023; Gustafsson et al., 2025; Newlands et al., 2024). For instance, a study by Jackman et al. (2023) reported that 10 min of HWI at 40°C did not change maximal strength. In another study measuring power, Solsona et al. (2023) demonstrated that 20 min of HWI at 41°C did not increase post-exercise power output. Gustafsson et al. (2025), observed no beneficial change on sprinting, jumping, or maximal strength following 20 min of HWI at 42°C. Consistent with this, Newlands et al. (2024) found that 25 min of HWI at 38.5-40°C did not enhance power following exercise. In contrast, studies employing prolonged exposures (> 30 min) have reported more favorable outcomes on the recovery of muscle function (Rodrigues et al., 2023; Cheng et al., 2017). Rodrigues et al. (2021) reported that immersion at 42 °C for 90 min increased muscle strength in human quadriceps muscles. Alternatively, higher water immersion temperatures (e.g. 43-46°C) for < 30 min duration have also been employed to assess the effects of HWI on muscle function. For example, De Ruiter et al. (1999) demonstrated in human hand muscles that elevating muscle temperature in a 45°C bath for 20 min accelerated rate of torque development (RTD) and half-relaxation time (HRT) of electrically evoked isometric contractions, but it did not increase maximal tetanic strength. Furthermore, Clarke et al., (1963) demonstrated that 10 min of HWI at 46°C increased the acute recovery of handgrip strength following fatiguing exercise. Altogether, these studies highlight that both the duration of HWI and the employed temperature can influence functional outcomes: 1) immersion durations lasting < 30 min and temperatures ≤ 42°C has no effect on muscle strength or power, and 2) higher HWI temperatures ≥42°C and/or longer HWI

durations can accelerate muscle contractile kinetics and it may or may not improve maximal isometric strength.

Only a limited number of studies have linked the changes in muscle contractile function to increases in intramuscular temperature with heating. Previous work by Davies and Young (1983) reported that a ~3°C rise in intramuscular temperature was sufficient to improve RTD and HRT in human hand muscles, building off earlier foundational evidence showing improved contraction velocity and altered force-velocity relationships with heating in human knee extensor and elbow flexor groups (Bergh & Ekblom, 1979; Binkhorst et al., 1977; Clarke et al., 1967). Furthermore, Sargeant (1987) demonstrated that a rise in intramuscular temperature from 36.6°C to 39.3°C in the quadriceps femoris muscle increased peak knee extensor force. Together, these studies suggest that an elevated muscle temperature appears to increase maximal strength as well as enhance skeletal muscle contractile kinetics. However, these classic experiments did not determine what is the minimum change in intramuscular temperature required to observe alterations in neuromuscular function, and whether the intramuscular temperature required to improve maximal voluntary isometric strength differs from that required to improve contractile velocity and peak power. Recently, we demonstrated during 1 h of 10°C CWI of the human dorsiflexor muscles that peak power was impaired by a lesser degree of intramuscular cooling than required to reduce maximal voluntary isometric contraction (MVC) torque (Malekzadeh et al., 2026). Furthermore, previous experiments exclusively heated rested muscle over prolonged durations, whereas they do not replicate how HWI is commonly applied to a muscle already pre-warmed by prior exercise (Cheng et al., 2017; Rodrigues et al., 2025).

Therefore, this study aimed to investigate the time- and intramuscular temperature-dependent changes in skeletal muscle function following HWI. We utilized a randomized crossover study design in which young healthy participants completed a 1 h HWI at 44°C of their lower leg. Participants were involved in two conditions: 1) 1 h HWI at 44°C (HWI-only), and 2) non-fatiguing exercise followed by 1 h HWI at 44°C (Ex + HWI) to mimic the post-exercise use of HWI. Throughout the 1 h HWI period, skin temperature, intramuscular temperature, and neuromuscular function were periodically assessed in the dorsiflexors. We hypothesized that: 1) acute HWI (<30 min) will not affect muscle function due to a limited increase in intramuscular temperature, 2) prolonged HWI (>30 min) will improve isotonic peak power but not MVC torque by increasing intramuscular temperature, and 3) any improvement in muscle function associated with a rise in intramuscular temperature will be reversed following a re-cooling intervention.

2.2 Materials and methods

Participants

Fourteen healthy, recreationally active individuals (8 M, 6 F) were recruited to partake in the study (n = 14). Participants were deemed recreationally active if they self-reported exercising a maximum of 2 days per week. Recruited participants were 23.0 ± 3.0 years of age with an average height of 171 ± 12 cm and weight of 77 ± 18 kg. All participants were free of any acute or chronic neurological, musculoskeletal, cardiovascular or orthopaedic limitations that could affect their neuromuscular performance. Participants were asked to refrain from strenuous physical activity for at least 24 hours before experimental trials and to not consume caffeine on the day of testing. All participants gave informed, written consent, and the study was approved by the local ethics committee on human research (York University Human Participants Ethics Review Committee).

Experimental overview

To evaluate the neuromuscular effects of HWI, this study required participants to visit the laboratory on 3-4 occasions, separated by a minimum of 48 hours. Up to two sessions were conducted for familiarization, where the participants were fully habituated to the experimental protocols (i.e., were able to achieve > 90% voluntary activation (VA) assessed via the interpolated twitch technique). Participants were also familiarized with the exercise protocol, which required participants to perform unloaded voluntary concentric dorsiflexions and plantarflexions through a full range of motion (ROM) paced to a metronome at 100 repetitions per minute. Following successful familiarizaiton, participants completed two experimental testing visits in a randomized order. The experimental visits consisted of: 1) 1 h HWI at 44°C (HWI-only), and 2) exercise followed by 1 h HWI at 44°C (Ex + HWI). Throughout the 1 h HWI intervention, skin temperature,

intramuscular temperature, neuromuscular function metrics (i.e., MVC, peak power, VA, electrically evoked 10- and 50 Hz torque, electrically evoked power) were tracked. These assessments were measured across seven distinct time points: baseline (0 min) and at 10, 20, 30, 45, and 60 minutes during the HWI intervention, with a final tracking period following a post-HWI re-cooling protocol designed to return muscle temperature back to baseline values (Fig. 1).

Apparatus set-up

Ankle joint torque, angular velocity, peak power, and position metrics were quantified using a HUMAC NORM™ isokinetic dynamometer (Computer Sports Medicine Inc., Stoughton, MA, USA). Data streams were acquired through a 16-bit analog to digital converter at a sampling rate of 500 Hz (Micro1401, CED, UK). All testing was restricted to the participants' non-dominant left leg, with participants seated (hip: 110° flexion; knee: 120° extension; with 180° referenced as full extension). The maximal dynamic dorsiflexion ROM was set to 30° of plantar flexion and 10° of dorsiflexion (40° total ROM). The dorsiflexion angle was fixed at 30° of plantar flexion during isometric neuromuscular function assessments, while isotonic contractions spanned the full 40° concentric window from 30° of plantarflexion to 10° of dorsiflexion. A 4-point seatbelt harness was used to minimize extraneous movements during neuromuscular assessments. Furthermore, non-elastic tightening straps were securely fastened over the left quadriceps, across the ankle, and the mid-portion of the foot to minimize extraneous movements.

Temperature recordings

Intramuscular temperature was continuously recorded using a tissue implantable thermocouple microprobe (Model IT-21; Physitemp, Clifton, NJ, USA), which was inserted ~2 cm into the muscle belly of the tibialis anterior using a 21-gauge catheter (21 x 0.8mm), as performed previously (Malekzadeh et al., 2026). The microprobe plug was connected to a data acquisition system (Model BAT-12; Physitemp, Clifton, NJ, USA), and secured at the insertion site using 3M medical-grade tape and pro-wrap to prevent displacement. Muscle temperature was recorded at baseline, immediately before HWI, every minute during HWI, and immediately following HWI. Skin temperature was simultaneously recorded using a surface temperature probe (Physitemp, Clifton, NJ, USA) placed on the skin directly over the intramuscular probe site. The skin temperature was recorded at regular intervals identical to intramuscular temperature as mentioned above.

Electrical stimulation

A clinical bar stimulating electrode (Model E.SB010, Digitimer North America, Fort Lauderdale, FL, USA) was used to electrically stimulate the peroneal nerve (i.e., immediately inferior to the fibular head) to evoke contractions of the primary ankle dorsiflexor muscle, the tibialis anterior muscle (TA). Optimal electrode placement was determined by palpating the fibular head and fine-tuning position using single twitches to maximize dorsiflexion torque output. The peroneal nerve was evoked with a single pulse (single 200-μs square wave pulses, 400 V) using a constant current stimulator (Model DS7R; Digitimer, Welwyn Garden City, UK). The current was increased by 5 mA until a plateau in twitch torque occurred (Malekzadeh et al., 2026). To ensure supramaximal activation of the muscle, the current was increased by 30%, and the resulting single

pulse current was used to determine %VA, and post-activation potentiation (PAP) (Cheng and Rice 2009; Malekzadeh et al. 2026).

Hot water immersion protocol

Participants were seated with their left leg enclosed in a plastic bag before submerging into a hot water bath at 44°C (De Ruiter et al., 1999). The leg was fully submersed up to the level of the tibial tuberosity. The plastic bag served to protect the leg from direct skin contact and potential discomfort from the hot water. The 44°C water temperature was chosen for the current study because it is a commonly from previous hot-water immersion studies (De Ruiter et al., 1999; Chaillou et al., 2022), and also it was deemed through pilot testing to represent the upper tolerable temperature limit for immersion of the leg over a 1 h duration and. Water temperature was monitored using a digital thermometer, and an 1100W thermal immersion circulator (Model 820, Wancle Corporation, Donguan, China), to maintain temperature at 44°C.

Experimental procedures

There were two experimental conditions for each participant: 1) HWI-only, and 2) Ex + HWI. During all HWI trials, the leg was immersed in water for a total 60 min duration, but periodically the leg was quickly removed from the water at 10, 20, 30, 45, and 60 min of immersion to assess neuromuscular function in the isokinetic dynamometer (Fig. 1). The neuromuscular function testing sequence involved a resting unpotentiated twitch followed by a 3s isometric MVC with interpolated twitch, followed by a resting potentiated twitch, and then a 10- and 50 Hz tetanus. Thereafter two ballistic isotonic contractions were performed at a load of 20% of MVC torque, which is a load that achieves peak power in the dorsiflexors (Power et al, 2016). In addition,

involuntary peak power was assessed using 50 Hz isotonic stimulation of the peroneal nerve. The isotonic load was equivalent to 20% of the participants MVC torque, matching the relative load used during voluntary isotonic power. The total duration of each neuromuscular testing sequence was ~20-30s, with an additional 30 s for transition time between ice bath and dynamometer. During the Ex +HWI condition, a metronome at 100 RPM was used to pace participants during unloaded voluntary dorsiflexion, with the aim to induce an exercise-induced increase in intramuscular heat generation without causing fatigue, as determined through pilot testing. This would allow us to determine the potential of exogenous post-exercise heating to alter neuromuscular function after the muscle is already warmed endogenously by exercise. For this condition, participants were seated in a chair with their leg unsupported by the dynamometer, performing repeated voluntary unloaded dorsiflexions for 10-20 min. Previous studies have shown that an exercise-induced increase in muscle temperature by 2-3°C eventually stabilizes after about 10–20 min of work (Davies & Young, 1983; Richards et al., 2025; Malekzadeh et al., 2026; Bishop, 2003; Silva et al., 2018; Saltin et al., 1968). Immediately after the exercise performed in the Ex + HWI condition, a neuromuscular testing sequence verified the absence of fatigue. After neuromuscular function testing, the participants' leg was placed in 44°C hot water for 60 min. To determine whether potential HWI-dependent changes in neuromuscular function were due to increased muscle temperature following the 1 h of HWI, a final control experiment was performed by re-cooling the leg with an ice bath back to its baseline physiological temperature. After this baseline temperature was reached with cooling, neuromuscular function was re-assessed.

Data analysis

All analysis was performed offline using Spike2 V.10.12a (Cambridge Electronic Design, Cambridge, UK). Isometric MVC torque was defined as the peak torque value during the plateau of maximal voluntary efforts. To assess the VA, using the interpolated twitch technique, the percentage of voluntary activation (VA%) was determined using the equation: VA% = (1 – interpolated twitch torque/resting twitch torque) x 100 (Gandevia, 2001). PAP was determined by calculating the percentage increase in potentiated twitch torque after the MVC torque compared with the non-potentiated twitch torque and multiplied by 100% (Power et al., 2012; Seitz et al., 2015). Peripheral muscle function was assessed by stimulating with 10 and 50 Hz tetani for 1.5 seconds using the initial single pulse current that achieved the plateau in twitch torque (Richards et al., 2025; Power et al., 2012; Power et al., 2010). Electrically evoked power was assessed by stimulating the peroneal nerve at 50 Hz using the same current applied during the 10 and 50 Hz tetanic contractions. Power was measured isotonically at a load equivalent to 20% of maximal voluntary contraction (MVC) torque, consistent with the approach used to assess voluntary peak power. Peak torque for 10 and 50 Hz stimulations was defined as the maximal value in Newton metres (N·m). The ratio of 10 Hz torque to 50 Hz torque is used to infer changes in intramuscular Ca^{2+} regulation where greater impairments to torque are seen at lower vs. higher frequencies (Richards et al., 2025). The RTD at 100 ms presents an optimal measuring point for assessing rapid activation of the dorsiflexor muscle (Dalton et al., 2023; Power et al., 2016; Andersen et al., 2006). The RTD was determined by measuring the torque at the 100 ms timepoint on the 50 Hz tetanic contraction. Torque onset (i.e., 0 ms) was set using the 2^{nd} pulse during the 50 Hz tetanus, with RTD being calculated at 100 ms (i.e., RTD = (torque at 100 ms – torque at 0 ms)/0.1 s) (Malekzadeh et al., 2026). Half relaxation time (HRT) of the 50 Hz was determined by measuring

the time it takes to reach 50% of the torque from the last evoked stimulation. Peak voluntary concentric isotonic power was measured as the highest value achieved during the two ballistic isotonic contractions (Power et al., 2010). Visual feedback was provided using Spike2 ™ V. 10 software (Cambridge Electronic Design Ltd., Cambridge, UK) to ensure that participants exerted maximal effort during the MVC and peak power contractions.

Statistical analyses

All statistical analyses were performed using GraphPad Prism software (v10.2.1, San Diego, CA, USA). A one-way repeated measures ANOVA was used to compare baseline values for all dependent variables (e.g., skin temperature, muscle temperature, and neuromuscular function) across the experimental conditions. To evaluate the effects of HWI on muscle function with time and experimental condition as the independent variables, a two-way repeated measures was conducted. This analysis assessed differences in muscle function between conditions (i.e., from pre) across time and between post- intervention recooling, respectively. Post hoc analyses for one-way and two-way ANOVA were conducted using Dunnet's and Tukey's test, respectively. Dunnett's test for one-way ANOVA was used to compare each condition against a control, and Tukey's test for two-way ANOVA, to assess all pairwise comparisons. All data were normalized and discrepancies in the data were excluded using a multivariable outlier analyses test with the ROUT (Q = 0.5%) method, with a D'Agostino & Pearson test then being performed again to test for normality. Data within the text and figures are represented as means ± SD. Significant differences were determined at $p < 0.05$. A maximum of n = 3 data points at 45 min of HWI was excluded for the pre- and postcontraction of the 50-Hz HRT and a maximum of n = 3 data points at 60 mins was excluded for the pre- and postcontraction of the 50 Hz RTD due to reflex responses during the contraction and relaxation phase of 50-Hz tetani. This exclusion was implemented post

hoc after identifying a consistent pattern of these reflex-related artifacts across some participants. Therefore, a mixed-effects model for repeated measures was used instead in these specific cases. Data within the text and figures are represented as means ± SD. Significant differences were determined at $P < 0.05$.

2.3 Results

Baseline measures

Baseline skin temperature, intramuscular temperature, MVC torque, peak power, voluntary activation, and electrically evoked low and high frequency stimulation parameters of the dorsiflexors are presented in Table 1. No significant differences in any of the baseline variables were observed between the experimental trials ($p < 0.05$; Table 1).

Skin and intramuscular temperature effects across time

We assessed skin and muscle temperatures prior to, and throughout the HWI intervention (Fig. 2A-B). Just prior to the HWI intervention, skin and intramuscular temperatures increased significantly for the Ex + HWI condition but not in the control (HWI-only) condition ($p < 0.05$), with skin temperatures reaching 32.8 ± 1.2°C for Ex + HWI, and intramuscular temperatures reaching 36.3 ± 0.6°C, respectively. The acute rise in muscle temperature during the Ex + HWI condition was achieved without fatiguing the muscle as reflected by the unchanged MVC torque as well as evoked 10:50 Hz torque ratio (i.e., baseline MVC torque = 32.4 ± 10.6 N·m vs. post-exercise MVC torque = 31.0 ± 10.0 N·m, $p = 0.99$; and baseline 10:50 Hz torque ratio = 0.52 ± 0.14 N·m vs. post-exercise 10:50 Hz torque ratio = 0.53 ± 0.14 N·m, $p = 0.99$). Following 10 min of HWI, skin temperature increased similarly across both conditions, and thereafter reaching a plateau between 10-60 min of HWI (Fig. 2A). After 1 h of HWI, skin temperature was ~37°C with no differences across conditions ($p = 0.9$) (Fig. 2A). For intramuscular temperature (Fig. 2B), the Ex + HWI condition maintained a higher value compared to the HWI-only condition during the first 30 min of immersion ($p < 0.05$). Thereafter, intramuscular temperature plateaued in both

conditions. No statistically significant differences were observed for intramuscular temperature between Ex + HWI and HWI-only at 45 min ($p = 0.08$) or 60 min ($p = 0.07$).

Voluntary measures across time during hot water immersion

There was no observable change in MVC torque (%) throughout 1 h of HWI in either condition ($p > 0.05$) (Fig. 3A). Notably, there was a significant increase in peak power (%) that occurred after only 10 min of HWI in both the HWI-only (111 ± 11.8%, $p < 0.05$), and Ex + HWI conditions (115.8 ± 17.7%, $p < 0.05$), respectively. Peak power remained elevated up to 45 min in HWI-only (109.9 ± 9.9%, $p < 0.05$) but only lasted up to 20 min in Ex + HWI (115.2 ± 12.4%, $p < 0.05$) (Fig. 3B). Voluntary activation (%) was near-maximal and did not decrease across any timepoint for either condition ($p = 0.49$) (Fig. 3C). To further illustrate the time-dependent changes in muscle function, we conducted a post-hoc comparison of peak power and MVC torque following the HWI-only condition. This analysis revealed a difference between peak power and MVC torque at each time point during the 1 h protocol ($p < 0.05$), further indicating that peak power was more improved and more sensitive to heating than MVC torque (Fig. 4B). Representative force and power tracings from a single participant further illustrate the effects of HWI on muscle function over time (Supplemental Fig. 1).

Involuntary measures across time during hot water immersion

Electrically evoked isotonic power increased in both the HWI-only and Ex + HWI conditions ($p < 0.05$). This increase was observed earlier at 10 min in HWI-only whereas in the Ex + HWI condition it increased at 20 min. Electrically-evoked power then remained elevated in both conditions up to 60 min of HWI (Fig. 4A). Low-frequency 10 Hz electrically evoked torque decreased following 10 min of HWI in both conditions (HWI-only 86.9 ± 14.3%, $p < 0.05$ vs. Ex = HWI 78.7 ± 23%, $p < 0.05$) (Fig. 5A). No changes in 50 Hz torque were observed throughout 1 h of HWI in both conditions (HWI-only:94.7 ± 19.3%, $p = 0.8$ vs. Ex + HWI: 99.8 ± 19.0%, $p = 0.8$) (Fig. 5B). Due to the reduction in 10 Hz torque at 10 min HWI without any change in 50 Hz torque at the same timepoint, we observed a decrease in 10:50 Hz ratio following 10 min of HWI (HWI-only: 0.51 ± 0.16 N·m, $p < 0.05$), and following 30 min of HWI, Ex + HWI (0.42 ± 0.12 N·m, $p < 0.05$), respectively (Fig. 5C). Contractile kinetics were assessed by measuring the 50 Hz RTD and HRT. In the HWI-only condition, the RTD increased after 20 min of HWI and remained elevated throughout the remainder of the 1 h HWI (119.4 ± 20.7 N·m, $p < 0.05$). However, in the Ex + HWI condition, RTD increased later at 30- 60 min of HWI (114 ± 13.2 N·m, $p < 0.05$) (Fig. 6A). The HRT of the 50 Hz tetani was different between conditions at the start of the intervention. At 0 min, HRT was lower in the Ex + HWI condition compared to HWI-only ($p = 0.047$; Fig. 6B), and this difference persisted at 10 min of immersion ($p = 0.049$; Fig. 6B. Between 10-60 min of immersion, 50 Hz HRT remained decreased in both conditions (Fig. 6B). In addition, there was no significant change in PAP for either condition at any time point throughout 1 h of HWI ($p > 0.05$) (Fig. 6C).

Post-HWI cooling of skin and intramuscular temperature on muscle function

Following the 1 h of HWI, we implemented a CWI protocol to determine whether returning muscle temperature back to its baseline temperature reverses the HWI-induced alterations in neuromuscular function (see Fig. 2A-6C). Following 60 min of HWI, employing CWI decreased skin temperature to 27.7 ± 2.4°Cin HWI-only ($p < 0.05$), and 27.4 ± 2.0°C in Ex +HWI ($p < 0.05$), respectively (Table 2). As expected, intramuscular temperatures returned back to baseline values after the CWI (HWI-only: 33.5 ± 0.57°C, $p = 0.81$ vs. Ex + HWI: 33.8 ± 0.7°C, $p = 0.97$) (see Table 2). MVC torque showed no change post-CWI in both conditions (Fig. 3A). The HWI-induced increase in peak power returned to baseline following CWI in HWI-only (99.3 ± 16.8%, $p = 0.99$), and in Ex + HWI (97.9 ± 25.5%, $p = 0.99$) (Fig. 3B). CWI did not alter %VA in either condition (HWI-only: 96.5 ± 3%, $p = 0.57$ vs. Ex + HWI: 97 ± 3%, $p = 0.53$). The contractile kinetics of the muscle such as involuntary peak power returned back to baseline values following CWI in both conditions (HWI-only: 106.5 ± 23.5%, $p = 0.96$, and Ex + HWI: 102.4 ± 14.0%, $p = 0.99$) (Fig. 4A). 50 Hz RTD returned back to baseline in HWI-only (105.5 ± 19%, $p = 0.79$) and in Ex + HWI (99.5 ± 25.2%, $p = 0.99$) (Fig. 6A). 50 Hz HRT returned to baseline in HWI-only (101.3 ± 16.7°C, $p = 0.99$) and Ex + HWI (97.0 ± 21.0°C, $p = 0.99$) (Fig. 6B). No observable change was reported on PAP following the CWI intervention in HWI-only (163.7 ± 54.7%, $p = 0.2$), and Ex + HWI (156.9 ± 38.6%, $p = 0.7$) (see Table 2).

2.4 Discussion

This study investigated the time- and temperature-dependent changes in skeletal muscle function of the dorsiflexors during prolonged HWI. Our primary findings demonstrate that just 10 min of 44°C HWI was sufficient to enhance peak power and 50 Hz HRT. Conversely, more prolonged exposures did not alter maximal isometric strength parameters, including MVC torque and 50 Hz electrically evoked torque. In mimicking post-exercise HWI, we also demonstrated that the exercise-induced increase in intramuscular temperature showed no apparent differences when compared to the benefits seen with passive heating (i.e., HWI-only condition). Furthermore, a control experiment involving a 1 h post-HWI re-cooling intervention confirmed that the heating-induced increases in peak power and contractile kinetics are intramuscular temperature-dependent.

We initially hypothesized that short duration HWI (<30 min) would exert minimal impact on neuromuscular function due to an insufficient rise in intramuscular temperature, whereas prolonged HWI (>30 min) would progressively enhance muscle function via markedly elevated tissue temperatures. Contrary to our hypothesis, our results revealed that peak power was enhanced with only 10 min HWI, and extending the heating duration to 1 h offered no additive benefits to peak power output. This early increase in peak power and subsequent plateau with continued HWI challenge the common view that 'more is better' regarding HWI duration (Rodrigues et al., 2025).A We also compared passive heating (HWI-only) against a non-fatiguing post-exercise protocol (Ex + HWI) simulating how this thermal intervention is typically performed by athletes (Bishop, 2003; McGowan, 2015; Richards et al., 2025; Ahokas et al., 2025). Our pre-HWI exercise protocol successfully raised baseline intramuscular temperature by ~3°C in the Ex +HWI condition. Interestingly, pre-warming the muscle with non-fatiguing exercise did not accelerate or

amplify the performance benefits more than what is seen with passive heating (HWI-only). This further suggests a metabolic threshold with heating towards improvements in peak power.

Our observation that passive HWI selectively benefits muscle explosiveness as well as contractile speed (i.e., power, 50 Hz RTD, 50 Hz HRT), and not maximal strength is a key novel finding of the current study. Although previous literature has suggested that acute heating might be insufficient to alter performance and that longer durations are required (Fuchs et al., 2020; Rodrigues et al., 2022; Rodrigues et al., 2023; Newlands et al., 2024), our data demonstrate that maximal strength remains entirely resistant to thermal intervention regardless of exposure time. Our finding aligns with the work of Binkhorst et al. (1977), who observed no changes in maximal isometric handgrip strength following 30 min of HWI at 39°C, spanning a broad temperature range (22.5 to 38°C). Consistent with the results of our current study, De Ruiter and De Haan (1999) reported that passive heating of the quadriceps muscles from ~34°C to 37°C improved the RTD and HRT during both electrically evoked and voluntary contractions yet they showed no change in maximal tetanic strength. One potential explanation for why a rise in intramuscular temperature may not influence maximal strength, as seen in previous studies is that during a maximal isometric contraction, all crossbridges within muscle fibres are already fully activated and contributing to force generation. Thus, an acute rise in intramuscular temperature is unlikely to yield further mechanical improvements toward enhancing maximal strength (i.e., ceiling effect) (De Ruiter & De Haan, 1999; Binkhorst et al., 1977).

While prior studies have often focused exclusively on either maximal strength or velocity (De Ruiter & De Haan, 1999; Binkhorst et al., 1977; De Ruiter et al., 1999), our results highlight the greater sensitivity of peak power to HWI. Power is the product of force and velocity, and it serves as a fundamental measure of skeletal muscle explosiveness (Hill, 1938; Ranatunga & Wylie,

1983; Power et al., 2016; Power et al., 2010). Prior studies that have looked at the effects of acute (<30 min) HWI at moderate temperatures (38.5 to 41°C) have shown that such protocols do not improve power output (Solsona et al., 2023; Newlands et al., 2024). Our novel findings demonstrated that 10 min of 44°C HWI was sufficient to elicit an 11% increase in voluntary peak power in the dorsiflexor muscles. While prior research shows that altering dynamic human performance may require a longer duration (45 to 90 min) (Rodrigues et al., 2023; Rodrigues et al., 2025), or more aggressive temperatures (46°C) (Clarke et al., 1963; Fuchs et al., 2020; Chaillou et al., 2022), our findings suggest that both the duration of HWI as well as the temperature employed are important in enhancing peak power, with HWI temperature >41°C being critical since previous studies employing immersion temperatures ≤41°C showed no change in power output .

Our involuntary measures of muscle function suggest that an intramuscular mechanism underlies the increase in peak power with HWI, such as optimized SR Ca^{2+} handling, enhanced myofilament sensitivity, or accelerating cross-bridge kinetics (Bigland-Ritchie et al., 1992; Kobayashi et al., 2005; Allen et al., 2008; Ranatunga, 2010). Unlike the low-frequency (10 Hz) stimulation, which decreased at 10 min, high-frequency (50 Hz) torque responses remained resistant to change across the timeline. This frequency-dependent difference likely reflects accelerated cross-bridge attachment and detachment kinetics under warmer conditions (Rall & Woledge, 1990; De Ruiter et al., 1999; Ranatunga, 2010; Richards et al., 2025). At high stimulation frequencies, accelerated cross-bridge cycling speeds up force fusion and optimizes peak tension. However, at low frequencies (10 Hz), an elevated intramuscular temperature can enhance relaxation kinetics which can then counteract force summation (De Ruiter et al., 1999; Westerblad et al., 1997; Ranatunga, 2010). Consequently, we noticed a decrease in the 10:50 Hz ratio at 10

min of HWI and although this ratio is traditionally associated with prolonged low frequency force depression or structural impairments in Ca^{2+} release, in a passive heating context, it may reflext an accelerated cross-bridge dissociation rate whereby the rate of torque development and relaxation is too rapid to summate (Allen et al., 2008; Cheng et al., 2017; Richards et al., 2025). This kinetic acceleration is further supported by our contractile tracking, where 50 Hz RTD and HRT shifts were rapidly detected within the first 10 to 20 min of HWI. Specifically, the rapid shortening of 50 Hz HRT at 10 min mirrored the exact timepoint at which voluntary peak power was maximized. On the other hand, 50 Hz RTD mirrored the timepoint at which involuntary peak power was increased at 20 min. This chronological coupling strongly suggested enhanced excitation-contraction coupling, such as accelerated SR Ca^{2+} handling, and faster myosin ATPase detachment rates may underpin the improvement in peak power with elevated intramuscular temperature (Godt and Lindley, 1982; Ranatunga, 2010; Kobayashi et al., 2005; Watanabe et al., 2018). Furthermore, we observed that HWI did not alter PAP across any time point. PAP reflects the acute enhancement of force following a conditioning contraction and can be an indication of myosin light chain phosphorylation which moves the myosin heads closer to the actin filaments to increase Ca^{2+} sensitivity (Bárány, 1967, Sweeney, 1993; Vandenboom et al., 1996; Hodgson et al., 2005; Blazevich & Babault, 2019). No effect was observed on PAP with prolonged HWI suggesting that myosin light chain phosphorylation was not a contributing mechanism behind the acute increase in peak power. Furthermore, partial limb immersion as employed in our study design selectively elevates local intramuscular temperature without imposing the systemic thermal strain, cardiovascular distress, or elevated core temperature associated with full-body (Thomas et al., 2006). Controlling core temperature prevents the central nervous system from reducing VA (Nybo,

2008; Todd et al., 2005), thereby allowing the intrinsic, peripheral benefits of local muscle heating (Rodrigues et al., 2023, Thomas et al., 2006).

Another novel aspect of our study design was the control condition where we returned the intramuscular temperature back to baseline values using a cooling intervention to verify whether the temperature dependent effects of HWI on voluntary and involuntary peak power as well as 50 Hz RTD and HRT could be reversed. Indeed, our results demonstrated that when local muscle temperature was fully restored to baseline values, the heat-induced improvements in peak power, involuntary peak power, 50 Hz HRT, 50 Hz RTD, and 10:50 Hz ratio were fully reversed. These novel findings confirm that the heating-induced improvements in peak power and muscle contractile kinetics were muscle temperature-dependent.

2.5 Conclusion

We have shown that only 10 min of 44°C HWI is required to improve peak power and contractile kinetics of the dorsiflexor msucles, whereas 60 min of HWI had no effect on maximal strength. Returning muscle temperature back to baseline values via CWI reversed the HWI-induced increases in voluntary and electrically evoked peak power as well as the increased contractile kinetics, confirming that the effects of HWI on contractile performance is temperature dependent. Together, these findings indicate that the effects of HWI are intramuscular temperature dependent.

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Baseline measurements

Baseline measures of intramuscular and skin temperature as well as neuromuscular function were assessed prior passive heating, or exercise (Table 1). There were no significant differences in the baseline variables at the start of the two experimental visits ($p > 0.05$, Table 1)

Table 1: Pre-intervention baseline measures of neuromuscular function

	HWI-only trial	**Ex + HWI trial**	One-way ANOVA
Muscle temperature	33.7 ± 0.4	33.9 ± 0.5	$p = 0.99$
Skin temperature	31.7 ± 0.7	31.7 ± 0.9	$p = 0.99$
MVC torque (N·m)	33.2 ± 9.3	33.9 ± 10.9	$p = 0.84$
Peak power (W)	34.2 ± 7.9	34.8 ± 9.5	$p = 0.87$
Voluntary activation (%)	98 ± 2.0	98.5 ± 3.0	$p = 0.61$
10 Hz torque (N·m)	11.9 ± 4.3	12.5 ± 5.8	$p = 0.82$
50 Hz torque (N·m)	20.0 ± 5.8	19.8 ± 7.4	$p = 0.90$
10:50 Hz torque ratio	0.61 ± 0.17	0.64 ± 0.19	$p = 0.99$
Involuntary peak power (W)	10.6 ± 3.5	10.7 ± 5.4	$p = 0.99$
PAP (%)	170.9 ± 73.1	154.3 ± 59.8	$p = 0.36$

Table 2: Muscle temperature restoration and neuromuscular function 1 hour post-HWI

Assessment	HWI-only 1 h	CWI	*p*-value	Ex + HWI 1 h	CWI	*p*-value
Skin temperature	36.9 ± 1.5	**27.8 ± 2.4***	$p < 0.05$	36.9 ± 0.6	**27.4 ± 2.0***	$p < 0.05$
Intramuscular temperature	38.9 ± 0.5	**33.5 ± 0.6***	$p < 0.05$	39.3 ± 0.3	**33.8 ± 0.7***	$p < 0.05$
MVC (%)	96.7.1 ± 5.9	93.3 ± 9.5	$p = 0.09$	97.9 ± 7.8	96.0 ± 8.1	$p = 0.3$
Peak Power (%)	108.2 ± 16.8	99.3 ± 16.8	$p = 0.051$	96.5 ± 29.4	90.3 ± 27.2	$p = 0.1$
10 Hz (%)	81.1 ± 14.8	85.5 ± 15.9	$p = 0.3$	85.3 ± 20.5	94.8 ± 27.2	$p = 0.058$
50 Hz (%)	94.7 ± 19.3	96.6 ± 18.6	$p = 0.5$	99.8 ± 19.0	100.9 ± 17.3	$p = 0.7$
PAP (%)	205.9 ± 113.0	163.7 ± 54.7	$p = 0.2$	162.7 ± 68.2	156.8 ± 38.5	$p = 0.7$
Involuntary Peak Power (%)	130.7 ± 29.6	**109.3 ± 23.7***	$p < 0.05$	136.1 ± 47.3	**110.2 ± 19.2***	$p < 0.05$

Restoring muscle temperature post 1 h HWI on muscle function. Data are shown as mean (± SD) (n = 14). *Significant difference between groups of any condition* (†, $p < 0.05$). *A significant temperature change over time compared to time 1 h* (*, $p < 0.05$).

Figure 1.

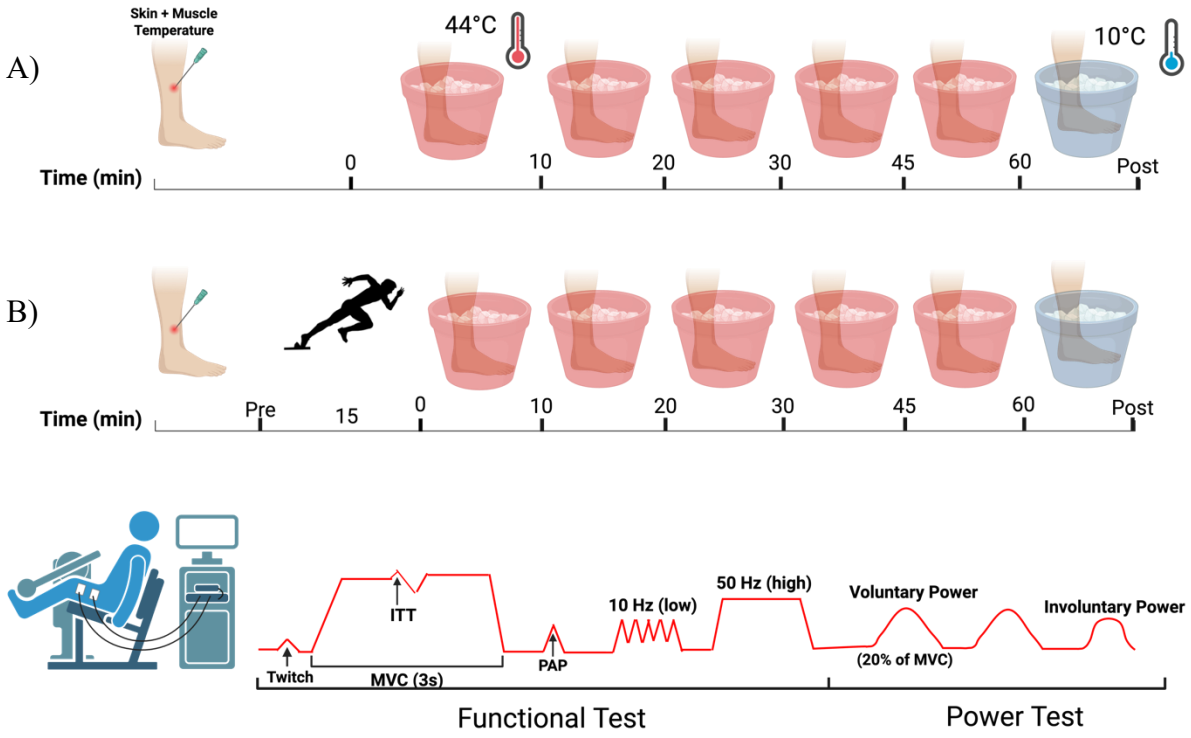


Figure 1: Schematic outlining the experimental timeline and procedures.

Study schematic representing an outline of the experimental procedures during A) HWI-only, and B) Exercise + HWI condition; Illustration created using Biorender.com.

Figure 2.

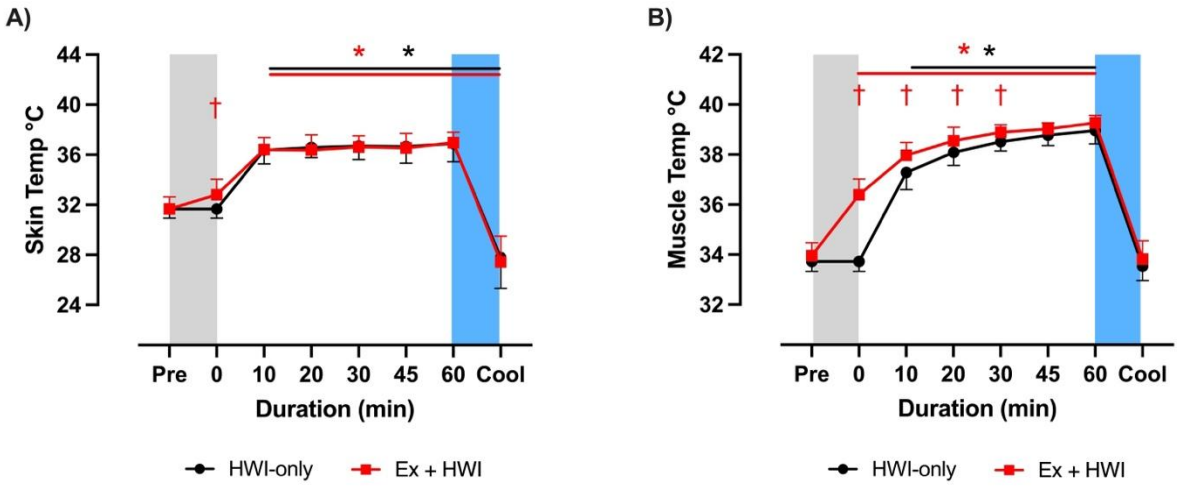


Figure 2: Time course changes in skin and intramuscular during HWI. Skin temperature (A), and intramuscular muscular temperature (B), were assessed continuously from Pre-intervention, to 60 min of HWI, and again post-HWI cooling. At 0 min, skin temperature in the Ex + HWI was greater than that in the HWI-only condition. Intramuscular temperature was significantly different between conditions at time 0- to 30 min of HWI, with Ex + HWI displaying a greater acute increase in muscle temperature. Following the HWI protocol a cooling intervention brought muscle temperature back to baseline values, while skin temperature decreased lower and this observation was significant. Data are shown as mean (± SD) (n = 14). Significant difference between groups of any condition († $p < 0.05$). A significant temperature change over time compared to time 0 (* $p < 0.05$).

Figure 3.

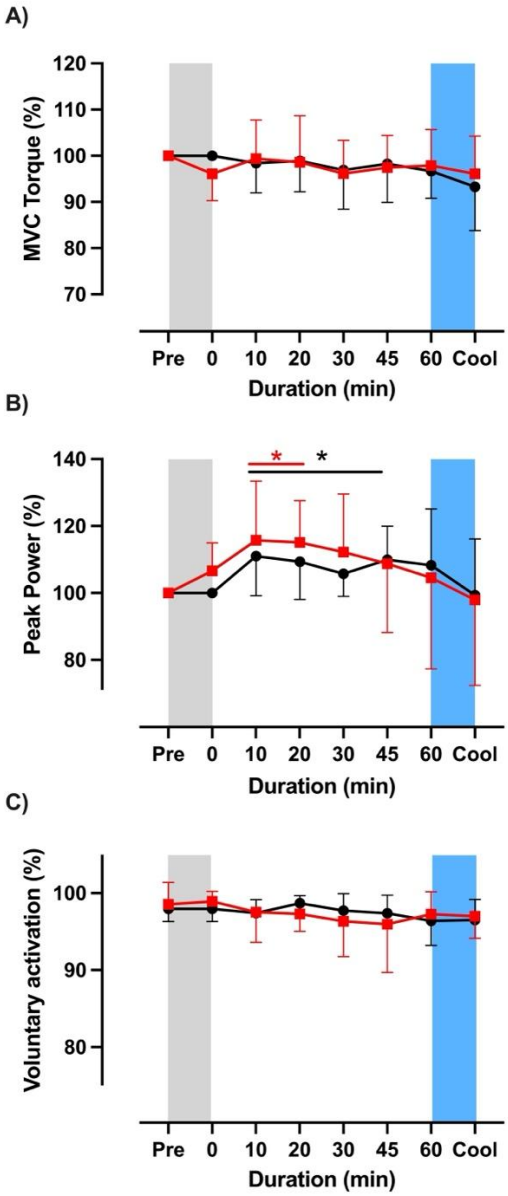


Figure 3: Time and temperature course changes in neuromuscular function. Maximal voluntary contraction (MVC) torque (%) (A), peak power (%) (B), voluntary activation (%) (C), between HWI-only, and Ex + HWI conditions were assessed at time Pre-, 0, 10, 20, 30, 45, and 60 min of HWI, and again following post-HWI cooling. A) MVC torque remained unchanged during HWI and Ex + HWI. B) Peak power (%) was significantly increased in both conditions similarly at only 10 min of HWI with no group differences seen, C) %VA was unaltered following both conditions. Data are shown as mean (± SD) data (n = 14). Significant difference between groups († $p < 0.05$). Significant differences over time (* $p < 0.05$).

Figure 4.

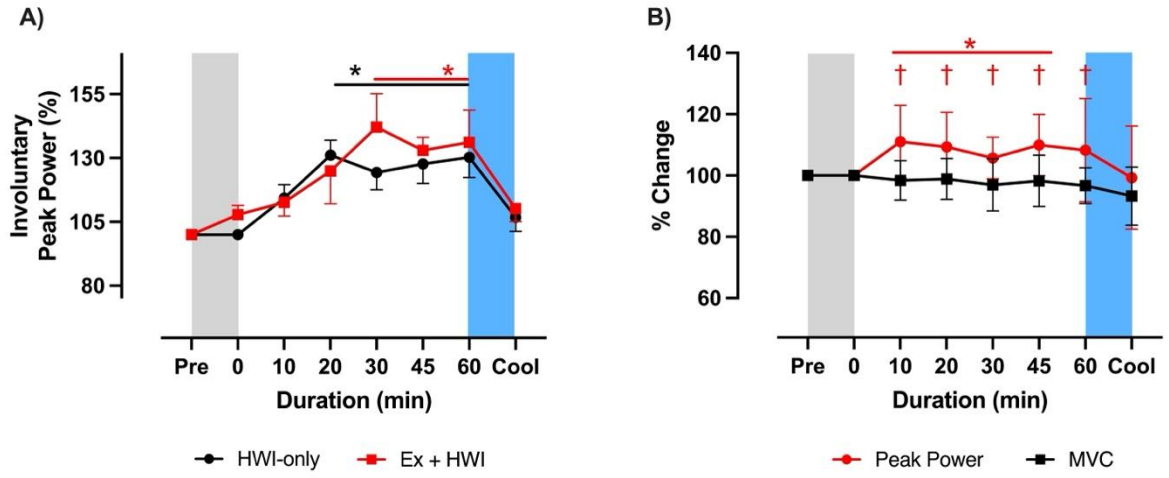


Figure 4: Time dependent changes in neuromuscular function during HWI-only. Involuntary isotonic power (%) (A), and a comparison between maximal voluntary contraction (MVC) torque (%), and voluntary peak power (%) across 60 min of HWI in the HWI-only condition at specified time points (Pre, 0, 10, 20, 30, 45, 60 min, and again post-cooling) (B). A) Involuntary power increased earlier in the HWI-only condition at 20- to 60 min, and was seen to increase later in the Ex + HWI at 30- to 60 min. B) peak power (%) significantly increased at 10- to 45 min, while MVC torque showed no changes during HWI in the HWI-only condition. Additionally, peak power (%) was significantly different from MVC torque at each time point during the 60 min of HWI. Post-HWI cooling intervention restored involuntary peak power A), and voluntary peak power (B) to baseline values. MVC (black squares) and peak power (red circles) are presented as mean ± SD (n = 14). Significant difference between groups († $p < 0.05$). Significant differences over time (* $p < 0.05$).

Figure 5.

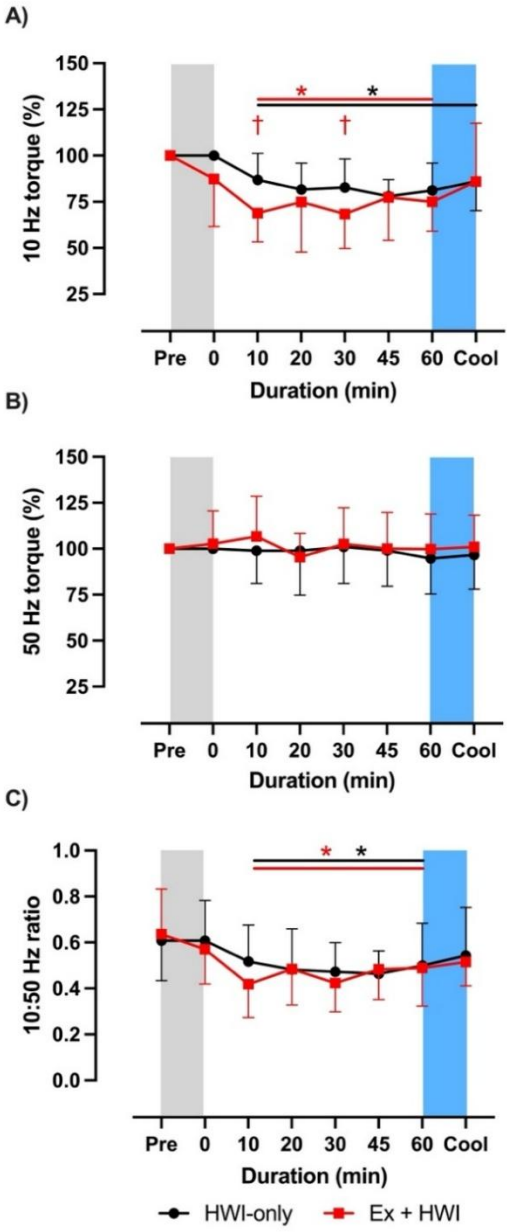


Figure 5: Time and temperature course changes in electrically evoked torque. 10 Hz low-frequency electrically evoked torque (%), 50 Hz high-frequency electrically evoked torque (%), 10:50 Hz ratio, between HWI, and Ex + HWI, conditions were assessed at Pre, 0, 10, 20, 30, 45, and 60 min of HWI, and again post- cooling a) 10 Hz torque (%) was significantly reduced across both conditions during the HWI. b) 50 Hz torque (%) was unaffected across the 60 min of HWI. c) 10:50 Hz ratio decreased at 10- 60 min for both HWI-only and Ex + HWI. Data are shown as mean (± SD) data (n = 14). Significant difference between groups († $p < 0.05$). Significantly differences over time (* $p < 0.05$).

Figure 6.

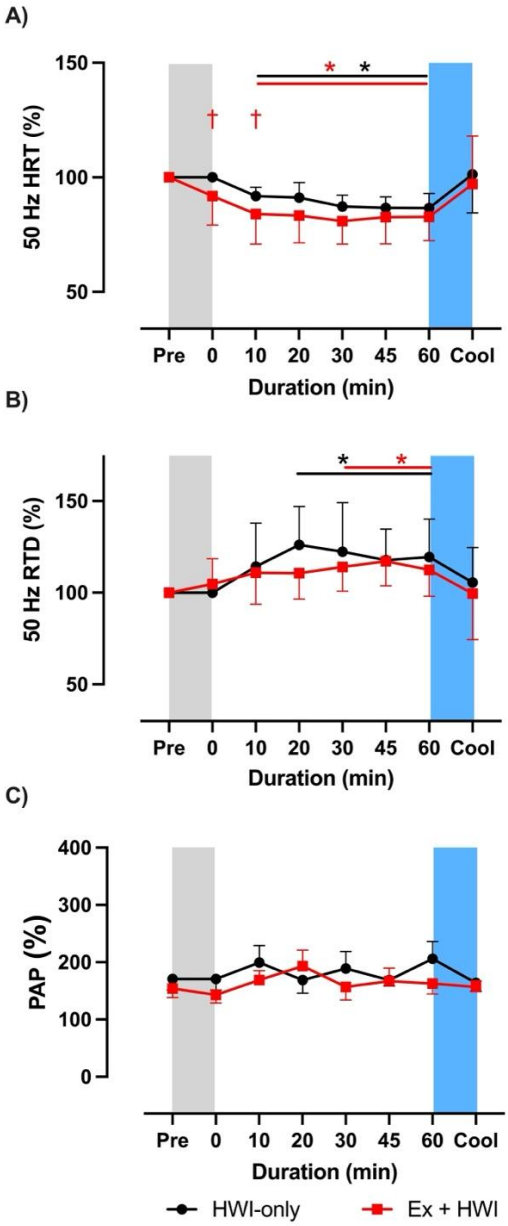
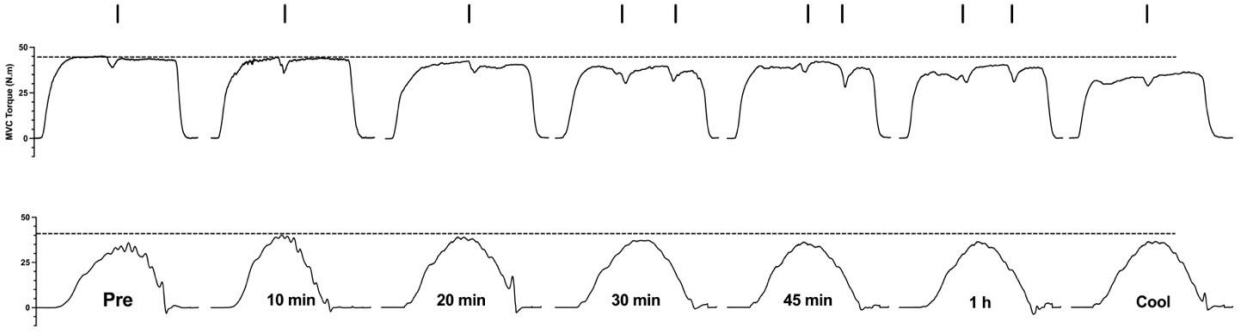


Figure 6: Time and temperature course changes in electrically evoked torque characteristics. 50 Hz RTD (%), 50 Hz HRT (ms), PAP (%), between HWI, and Ex + HWI conditions were assessed at Pre, 0, 10, 20, 30, 45, and 60 min of HWI and again post- cooling. a) 50 Hz HRT (%) was decreased from 10- to 60 min with a slightly greater drop at time 0- to 10 min during Ex + HWI. b) RTD (%) was increased following 20 mins of HWI in HWI-only and at 30 min in Ex + HWI. c) PAP (%) remained unchanged during each condition. Data are shown as mean (± SD) data (n = 14). Significant difference between groups († $p < 0.05$). Significant difference over time (* $p < 0.05$).

Supplemental Figure 1.



Supplemental Figure 1: Original tracings from a representative participant during HWI-only. Illustration depicting the time-dependent changes in maximal voluntary strength and power throughout HWI. MVC torque and peak power traces are shown at baseline (PRE) during the 1 h HWI intervention and following a cooling protocol at the very end. The black vertical line just above the maximum voluntary contraction (MVC) torque traces indicate the timepoint in which the interpolated twitch was superimposed during the MVC. Reference lines were drawn at the peaks of baseline MVC torque and peak power to demonstrate the reduction in muscle function across each interval of HWI.

APPENDICES

Appendix A: Ethics Approval

YORK
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UNIVERSITY

Certificate #:	**e2024-195**
Initial Approval:	**08/01/24-08/01/25**
Amendments:	**Amendment approved: 10/07/25**
Renewals:	**10/07/25-10/07/26**
Current Approval Period:	**10/07/25-10/07/26**

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ETHICS RENEWAL - AMENDMENT APPROVAL

To: **Professor Arthur Cheng**
Department of Kinesiology & Health Science
Faculty of Health
ajcheng@yorku.ca

From: Alison M. Collins-Mrakas, Director, Research Ethics
(on behalf of Gillian Parekh, Chair, Human Participants Review Committee)

Date: Tuesday, October 7, 2025

Title: **Effects of warm water immersion and training status on post-exercise skeletal muscle recovery following sprint-interval exercise**

Risk Level: ☒ Minimal Risk ☐ More than Minimal Risk

Level of Review: ☒ Delegated Review ☐ Full Committee Review

With respect to your research project entitled, **"Effects of warm water immersion and training status on post-exercise skeletal muscle recovery following sprint-interval exercise",** the committee notes that, as there are no substantive changes to either the methodology employed or the risks to participants in and/or any other aspect of the research project, a renewal of approval re the proposed amendment(s) to the above project is granted.

Any further changes to the approved protocol must be reviewed and approved through the amendment process by submission of an amendment application to the HPRC prior to its implementation.

Ongoing research – research that extends beyond one year – must be renewed prior to the expiry date.

Any adverse or unanticipated events in the research should be reported to the Office of Research ethics (ore@yorku.ca) as soon as possible.

For further information on researcher responsibilities as it pertains to this approved research ethics protocol, please refer to the attached document, **"RESEARCH ETHICS: PROCEDURES to ENSURE ONGOING COMPLIANCE".**

Should you have any questions, please feel free to contact me at: acollins@yorku.ca.

Yours sincerely,

Alison M. Collins-Mrakas M.Sc., LLM
Director, Office of Research Ethics

Appendix B: Informed Consent Form

Informed Consent Form

Date: 2026-06-04

Study Name: Effects of warm water immersion and training status on post-exercise skeletal muscle recovery following sprint-interval exercise

Researchers:

Arthur J. Cheng, Ph.D. (Principal Investigator), Farquharson Room 351, Contact: 416-736-2100 ext. 30030
Andrew Richards, Farquharson Room 350, Contact: 416-736-2100, ext. 33160
Rohin Malekzadeh, Farquharson Room 350, Contact: 416-736-2100, ext. 33160
Alireza Vaziri, Farquharson Room 350, Contact: 416-736-2100, ext. 33160
Sophie Portnoy, Farquharson Room 350, Contact: 416-736-2100, ext. 33160
Parsa Ebrahimpoor, Farquharson Room 350, Contact: 416-736-2100, ext. 33160
Raaj Dudani, Farquharson Room 350, Contact: 416-736-2100, ext. 33160

Purpose of the Research:

High-intensity exercise leads to muscle fatigue which is typically exhibited as a rapid reduction in muscle strength or power. On the other hand, recovery of muscle strength and power in the post-exercise period can be a slow process that can take hours or even days. The functional implications of a slow recovery are that muscle fatigue may still persist at the start of the next days' exercise session, leading to exacerbated fatigue and impaired repeated bout exercise performance. Thus, it has been of great interest by athletes to find interventions that can potentially accelerate skeletal muscle recovery. The potential cause of the muscle fatigue and slow recovery is not completely known but is likely dependent on the muscle metabolic rate, primarily driven by mitochondria, which are the major energy producers within muscle. Intramuscular temperature is a critical regulator of muscle metabolic rate, with a higher temperature increasing and lower temperature decreasing metabolism. Warm water immersion of exercised muscles has emerged as a popular recovery intervention deemed to accelerate skeletal muscle recovery, although there is little scientific evidence to confirm its effectiveness as a recovery method. The purpose of this study is to investigate whether warm water immersion of their limb (i.e., submerged up to their knee) affects recovery of muscle function after exercise. This information can help us understand a fundamental aspect of muscle health and will be valuable when designing interventions to improve skeletal muscle recovery after exercise.

If you consent to participate in this research, you will be one of 100 participants.

Inclusion requirements: In order to participate in this study, you must be between the ages of 18 and 45 years old who are either endurance-trained (> 1 h of aerobic exercise per week) or not endurance-trained (< 1 h of aerobic exercise per week). You must agree to complete a standard medical questionnaire.

Exclusion requirements: You are not eligible to participate in or will be released from this study if you have any one of the following conditions: smoker or user of tobacco products, heart disease, rheumatoid arthritis, diabetes, poor lung function, uncontrolled high blood pressure (hypertension), taking aspirin or anticoagulant medication (blood thinners), or any health conditions that might put you at risks for this study.

What You Will Be Asked to Do in the Research:

Study Design:

You will visit the lab on 11-12 separate occasions as shown below:

Visit 1 (and 2): familiarization trial (NOTE: 2nd familiarization trial is only required if further habituation to the exercise test is needed).

Visit 3: exercise + 8h post-exercise recovery period muscle function assessment

Visit 4: 24h post-exercise recovery period muscle function assessment
Visit 5: exercise + 8h post-exercise recovery period muscle function assessment
Visit 6: 24h post-exercise recovery period muscle function assessment
Visit 7: Warming without exercise
Visit 8: Easy exercise followed by warming
Visit 9: Thermoneutral without exercise
Visit 10: Easy exercise followed by thermoneutral
Visit 11: Warming followed by exercise + blood flow occlusion
Visit 12: Thermoneutral following by exercise + blood flow occlusion
Visit 13: Easy exercise followed by cold water immersion + 3h recovery period muscle function assessment

All warm water immersion protocols will involve immersion of the limb up to the level of their knee in 40-50°C water. All thermoneutral water immersions will involve immersion of the limb to the level of the knee in 33-34°C. (i.e., matching physiological temperature).

At the start of the experimental sessions, a small intramuscular temperature probe will be inserted mid-depth into the belly of your dorsiflexor muscle to record intramuscular temperature. Thereafter, a gel-coated bar electrode will be placed on the skin overlaying the dorsiflexor and plantar flexor muscles. A single gel-coated electrode will be placed at the fibular head of the lower leg, and will be used to perform electrical stimulation of the muscle to test involuntary muscle strength. The stimulation intensity will be adjusted such that the electrically stimulated force will not exceed 60% of your maximum voluntary force. Additional gel-coated electrodes will be placed on the tibialis anterior muscle and soleus muscle for passive recording of muscle electrical activity and hair will be shaved in these locations to ensure no interference in the recording of the electrical activity by the electrodes. Alcohol swabs will be used to clean the skin of oils to ensure that the electrodes remain in contact with the skin. Body composition assessment of skeletal muscle mass will be evaluated using ultrasonography, which has shown to be as accurate and reliable as gold standard measures of muscle thickness (Paris et al., 2017). Ultrasound imaging will be used to measure muscle thickness and cross-sectional area of the dorsiflexor muscle at the site of the muscle belly of the tibialis anterior prior to intramuscular temperature insertion in visits 11, 12, and 13 (Paris et al., 2017, Paris et al., 2020). All ultrasound measurements will be taken at pre-defined landmarks, which are identified using bony-landmarks. All measurements will be taken in a supine posture on a padded table. Near infrared spectroscopy (NIRS), is a non-invasive tool used to continuously measure muscle oxygenation and hemodynamics (McNeil et al., 2015). NIRS will be placed on the muscle belly of the tibialis anterior and will be taped onto the surface of the skin during visits 11, 12 and 13. Blood flow occlusion cuff will be used to differentiate between measures of improved blood flow and muscle oxygen extraction. The occlusion cuff will be placed just above the knee of the lower limb and inflated post intervention and immediately following exercise in visits 11 and 12.

During the first and second visits, the purpose here is to familiarize you with all experimental procedures.

During the third, fifth visit, the exercise protocol will be identical and will involve maximal effort dynamic dorsiflexion contractions (i.e., dorsiflexion is an ankle joint action that involves lifting your toes) at a load no greater than 50% of your maximum isometric strength with approximately 3-5 min rest between sets. After exercise cessation, the post-exercise recovery period will involve placing your exercised limb in a warm water bath, or the limb will be resting at room temperature without any water bath immersion. After this recovery period, you will repeat the exercise. The only difference between the experimental protocol in the second and third visit will be the post-exercise recovery intervention. The order of the recovery intervention will be randomized between the third and fifth visit. During the post-exercise recovery period, subjects will be periodically asked to briefly perform voluntary contractions in order to assess the recovery of muscle strength and power, and muscles will be electrically stimulated at these same timepoints to determine the recovery of involuntary muscle strength. As previously performed with the initial exercise, an exercise test will be performed during the post-exercise recovery period involving the same maximal effort dynamic dorsiflexion contraction protocol.

In the seventh visit, participants will have their muscles submerged in warm water for up to a 2h period without any prior exercise, and involuntary and voluntary dorsiflexion strength will be assessed prior to the warming, and at periodic intervals during the warming period and up to 1h after the warming period.

In the eighth visit, participants will be performing repeated unloaded dynamic dorsiflexion contractions and thereafter their muscles will be in warm water for up to a 2h period without any prior exercise. Involuntary and voluntary dorsiflexion strength will be assessed prior to the warming, and at periodic intervals during and after the warming period.

In the ninth visit, participants will have their muscles submerged in thermoneutral water for up to a 2h period without any prior exercise, and involuntary and voluntary dorsiflexion strength will be assessed prior to the thermoneutral, and at periodic intervals during the thermoneutral period and up to 1h after the thermoneutral period.

In the tenth visit, participants will be performing repeated unloaded dynamic dorsiflexion contractions and thereafter their muscles will be in thermoneutral water for up to a 2h period without any prior exercise. Involuntary and voluntary dorsiflexion strength will be assessed prior to the thermoneutral, and at periodic intervals during and after the thermoneutral period.

In the eleventh and twelve visit, participants will have their muscles submerged either in warm water or in thermoneutral water for up to a 2h period, which will be immediately followed by dynamic dorsiflexion exercise at a load not exceeding 50% of maximal voluntary strength (i.e., as performed in the third and fifth visits). A blood flow occlusion cuff will be placed on the lower limb (above the knee) and inflated at set time points before and after the water immersion intervention and after exercise to assess measures of isolated limb blood flow and muscle oxygen extraction using near-infrared spectroscopy (McNeil et al., 2015).

Lastly, in the thirteenth visit, participants muscles will be submerged in cold water (10°C) for up to a 1h period immediately following exercise, achieving a 10°C decrease in intramuscular temperature below the physiological temperature as previously performed (Malekzadeh et al., 2026 Med Sci Sports Exerc). Thereafter the leg will be removed from the cold water and muscle function will be assessed over a 3h recovery period.

In the experiments involving warm water immersion, when the muscles are removed from the warm water, the leg will be briefly cooled with ice-chilled water to bring the skin and muscle temperature back to physiological temperature to assess how skin temperature vs. intramuscular warming affects neuromuscular function.

Location: Unless otherwise stated, all experimental trials will be completed in the Farquharson Life Sciences Building Room 322 at York University.

Time Commitment and Attire: This study will involve a maximum of 46 hours of your time over ten-eleven trials. Please come to the laboratory (Farquharson Building, Room 322) with appropriate attire for exercise (i.e., shorts, t- shirt, low socks, gym shoes)

Renumeration: Participants will not receive any compensation for their involvement in this research study. Your participation will provide scientists with valuable information regarding the role of muscle temperature manipulation on post-exercise recovery from skeletal muscle fatigue.

Dietary requirements: All participants will eat as they always do. Subjects are asked to refrain from consuming caffeine on the day of experiments.

Recreational activity restrictions: You will be required to refrain from all other forms of exercise starting from 24 hours prior to your visit to the lab. This includes intramurals, weights and endurance exercise at the gym and team sports. Exceptions may be made if you walk or cycle to work but this must be discussed with the investigators. This is true for all groups in this study. Since the purpose of our experiment is to study muscle fatigue and recovery, prior exercise can affect the measurements being made in the muscle during the experiment.

Risks and Discomforts:

Here we have identified the risks which might be experienced by the subject by being engaged in this experiment, and procedures by which we will manage these risks. Should the subject feel uncomfortable with any aspects of the experiment, they are free to withdraw from participation at any timepoint. Nonetheless, we have made measures to minimize and manage the physical and psychological/emotional risk potentially experienced in this study based on the following mentioned procedures. Minimal risk is defined as "research in which the probability and magnitude of possible harms implied by participation in the research is no greater than those encountered by participants in the aspects of their everyday life that related to the research":

Intramuscular temperature probe (more than minimal risk): The risks of using an intramuscular temperature probe are considered minimal but to reduce the risk of infection, the experimenters will be responsible for installing the temperature probe on participants under sterile conditions. Please refer to the attached form entitled "Description of Medical Procedures" for a complete description of the medical procedures to be performed during the study and the potential risks and discomforts associated with these procedures

Exercise (minimal risk): Potential physical (muscle) discomfort can arise from the exhaustive physical exercise being performed on the dorsiflexor muscle group, but this would be no different than that experienced following a fatiguing exercise session in the gym. Furthermore, the subject may also experience delayed onset muscle soreness in the days following the exercise session, but this is a natural process that follows unaccustomed physical activity and would be no different than that experienced following an exercise session in the gym. **Risk management**: The physical muscle fatigue and soreness related to the exercise are unavoidable. However, regarding tolerability of the exercise, the familiarization exercise session will habituate subjects to the exercise protocol by gradually increasing the exercise intensity.

Electrical stimulation (minimal risk): electrical stimulation can elicit some physical discomfort which is mostly sensed at the level of the skin. **Risk management**: to minimize potential discomfort, the stimulation intensity will be gradually increased to habituate subjects to the stimulation. The intensity of stimulation will only be adjusted up to a level that the subjects' can comfortably tolerate.

Near infrared spectroscopy (minimal risk): minimal risks may include discomfort at the site of sensor placement due to the adhesive used to secure the device. Near infrared spectroscopy (NIRS) is a non-invasive technique that will be used to monitor muscle oxygenation and blood flow through sensors placed on the surface of the skin continuously throughout a visit.Blood flow occlusion (minimal risk): potential physical (muscle) discomfort can arise from the application of blood flow occlusion. The occlusion will reach maximal pressure (300 mmHg) via a rapid inflation cuff pre-intervention/exercise, and post- intervention/exercise and maintained for up to 5 mins. Participants may experience sensations of pressure, tightness, or discomfort in the occluded limb, which are transient and resolve upon cuff deflation.

Ultrasound (minimal risk): minimal risks may include mild, temporary skin irritation from the ultrasound gel and slight discomfort due to light pressure from the transducer. Ultrasound is a non-invasive imaging technique used for assessing muscle thickness and cross-sectional area.

Warm water immersion (minimal risk): The warm water may induce skin discomfort during water immersion. **Risk management:** Skin damage will be prevented by placing the subjects' leg in a plastic bag to prevent direct contact of the water with the skin.

Cold water immersion (minimal risk): The ice-chilled water may induce skin discomfort during water immersion. **Risk management:** To reduce the risk of cold-related skin irritation, the participant's limb will be covered with a protective plastic barrier to limit direct skin contact while allowing controlled cooling. Participants will be continuously monitored, and verbal check-ins will be performed regularly to assess tolerance. In addition to potential skin irritation, cold water immersion may result in transient cold-induced pain, numbness, tingling, reduced muscle strength, decreased coordination, and temporary changes in sensation. With prolonged exposure, participants may also experience stiffness, delayed nerve conduction, and mild shivering. These effects are expected to be temporary and resolve upon rewarming. Participants may withdraw their limb from the chilled water at any time without penalty. The procedure will be terminated immediately if the participant experiences excessive discomfort or any adverse symptoms.

Psychological risks (minimal risk): Participants may feel embarrassed on their exercise performance. **Risk management:** The investigators will reassure each subject and make them feel as comfortable as possible within the laboratory. The doors will be closed for the entirety of testing to make the testing environment private.

Benefits of the Research and Benefits to You:
There will be no renumeration for your participation in this study. Your participation will provide scientists with valuable information regarding the role of muscle temperature manipulation on post-exercise recovery from skeletal muscle fatigue.

No Compensation for Injury: If, in the event that you are injured as a direct result of taking part in this study, you will be referred for appropriate treatment. No funds have been set aside to compensate you in the event of injury or illness related to this study and financial compensation for such things as lost wages, disability or discomfort due to such injury is not routinely available. You are not waiving your legal rights by signing the consent form, nor are you absolving the investigators from their legal or professional responsibilities.

Voluntary Participation and Withdrawal: Your participation in the study is completely voluntary and you may choose to stop participating at any time. Your decision not to volunteer, to stop participating, or to refuse to answer particular questions will not influence the *treatment you may be receiving, the nature of the ongoing relationship you may have with the researchers or study staff, or the* nature of your relationship with York University either now, or in the future. In the event you withdraw from the study, all associated data collected will be immediately destroyed wherever possible. *Should you wish to withdraw after the study, you will have the option to also withdraw your data up until the analysis is complete.*

Confidentiality: Unless you choose otherwise all personal identifying information you supply during the research will be held in confidence and unless you specifically indicate your consent, your name will not appear in any report or publication of the research.

The principal investigator will keep a link that identifies you to your coded information, but this link will be kept secure and available only to the principal investigator and/or selected members of the research team. Any information that can identify you will remain confidential.

Data will be collected on the dynamometer and computers, and notes will be taken on paper for data collection purposes. Your data will be safely stored in a locked facility [I.e., locked cabinet in Dr. Cheng's office] and only research staff/research team members will have access to this information. The completed non-identifiable data set used to generate papers for publication will be stored on Dr. Cheng's password-protected office computer. The data will be retained for a minimum of 10 years to comply with journal requirements. If requested by the journal, anonymized data will be submitted to a data repository. Confidentiality will be provided to the fullest extent possible by law.

Please note that at the end of the study de-identified data may be deposited into one or more publicly accessible scientific repositories, such as York University Dataverse, an institutional research data repository, managed by York University Libraries and provided by Scholars Portal on behalf of the Ontario Council of University Libraries (OCUL), through which researchers from around the world will have access to these data for future research, through a [CC, CC-BY, CC-BY-NC, or other] standard data sharing license. York University Dataverse does NOT accept content that contains confidential or sensitive information. Dataverse can be used to share de-identified and non-confidential data only. Contributors are required to remove, replace, or redact such information from datasets prior to upload. Scholars Portal makes backup copies of the uploaded data regularly in the event of a server or system malfunction, malicious attack, or other technical issues.

Questions About the Research? If you have questions about the research in general or about your role in the study, please feel free to contact Dr. Arthur Cheng either by telephone at (416) 736-2100, extension 30030 or by e-mail (ajcheng@yorku.ca). This research has received ethics review and approval by the Human Participants Review Sub-Committee, York University's Ethics Review Board and conforms to the standards of the Canadian Tri-Council Research Ethics guidelines. *If you have any questions about this process, or about your rights as a participant in the study, please contact the Office of Research Ethics, York University (e-mail ore@yorku.ca).*

Legal Rights and Signatures:

I (_____), consent to participate in "Effects of hot water immersion and training status on post-exercise skeletal muscle recovery following sprint-interval exercise". I have understood the nature of this project and wish to participate. I am not waiving any of my legal rights by signing this form. My signature below indicates my consent.

Visit 1: Signature _____ **Date** _____
Participant

Visit 1: Signature _____ **Date** _____
Principal Investigator

Visit 2: Signature _____ **Date** _____
Participant

Visit 2: Signature _____ **Date** _____
Principal Investigator

Visit 3: Signature _____ **Date** _____
Participant

Visit 3: Signature _____ **Date** _____

Visit 4: Signature _____ **Date** _____
Participant

Visit 4: Signature _____ **Date** _____
Principal Investigator

Visit 5: Signature _____ **Date** _____
Participant

Visit 5: Signature _____ **Date** _____
Principal Investigator

Visit 6: Signature _____ **Date** _____
Participant

Visit 6: Signature _____ **Date** _____
Principal Investigator

Visit 7: Signature _____ **Date** _____
Participant

Visit 7: Signature _____ **Date** _____
Principal Investigator

Visit 8: Signature _____ **Date** _____
Participant

Visit 8: Signature _____ **Date** _____
Principal Investigator

Visit 9: Signature _____ **Date** _____
Participant

Visit 9: Signature _____ **Date** _____
Principal Investigator

Visit 10: Signature _____ **Date** _____
Participant

Visit 10: Signature _____ **Date** _____
Principal Investigator

Visit 11: Signature _____ **Date** _____
Participant

Visit 11: Signature _____ **Date** _____
Principal Investigator

Visit 12: Signature _____ **Date** _____
Participant

Visit 12: Signature _____ **Date** _____
Principal Investigator

Visit 13: Signature _____ **Date** _____
Participant

Visit 13: Signature _____ **Date** _____
Principal Investigator

1. Consent to data deposit

I understand that my de-identified data will be placed into an open research data repository. Y / N

Signature _____ **Date** _____
Participant Name:

DESCRIPTION OF MEDICAL PROCEDURES

The study in which you are invited to participate involves the installation of an intramuscular temperature probe. Prior to any involvement, you are asked to read this form, which outlines the potential medical risks inherent to this procedure. In addition, you must also complete the attached "**2024 Par-Q+ Screening Questionnaire**", which is designed to identify any medical reason that might preclude your participation.

Intramuscular Temperature Probe Installation Procedure

The experimenters will perform the intramuscular temperature probe installation procedure. The temperature probe is thin wire of <1mm diameter. Using a disposal catheter, the temperature probe will be inserted through the skin and into the tibialis anterior (i.e., dorsiflexor muscle at the front of your shin) at a mid-depth of ~1.2 cm into the muscle belly upon which the disposal catheter will be removed and the temperature probe will remain in the muscle during visits 3,5,7,8). The temperature probe will only be installed in the tibialis anterior muscle on four of the eight visits.

Detailed description of the Temperature Probe Procedures and potential risks:

First, the area over the skin where the intramuscular temperature probe will be installed will be cleaned with an antiseptic solution. Second, the experimenter will install the intramuscular temperature probe using a disposable catheter, which will be used to quickly pierce the skin and insert the temperature probe into the muscle. During the time that the catheter is initially inserted (~1-2 sec), the sensation of pressure on the skin is felt, and on some occasions, this is moderately uncomfortable for a few seconds if the area of the skin where the sensor has been installed is sensitive. However, the discomfort passes quickly and the participant will be able to normally move their ankle and toes without discomfort.

Potential Risks: Intramuscular temperature probes are widely used in experimental research, and complications are rare provided that proper precautions are taken. However, there is a risk of infection at the site of the intramuscular temperature probe installation. However, this risk is virtually eliminated through proper cleansing of the area prior to the installation of the temperature probe. If you are in any way concerned about swelling and redness, please contact us immediately.

You will be excluded from participation if you are taking aspirin or any anticoagulant medication ('blood thinners') in order to avoid additional risks of bleeding complications.

The possibility of a medical emergency. In the unlikely event of a medical emergency, Dr. Robert Laham is fully aware of the procedure and the whole initiative in general. He is on call and willing to address any unexpected emergency. Dr. Robert Laham can be reached at Appletree Medical Centre, York Lane Malls, York University through a direct line at 416-473-3428 (or 416 479-8649 or 647-722-2370). The investigators will follow standard York Emergency Procedures if Dr. Laham cannot be reached at his direct line. Specifically, 911 will be called directly (9-911 from a local university extension) followed by calling Security Control Centre at Ex. 33333 for assistance.