Cellular, molecular and physiological effects of post-resistance exercise
cold water immersion: Implications for subsequent performance

Llion Arwyn Roberts
MSc, BSc (Hons)

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School of Human Movement Studies
Abstract

The objective of this thesis was to examine the efficacy of cold water immersion as a recovery therapy after resistance exercise. To achieve this objective, the research had two distinct aims, (1) to investigate how cold water immersion influences acute (short-term) physiological and performance responses following a single bout of resistance exercise, and (2) to investigate how regular (longer-term) use of cold water immersion following resistance training sessions may effect training-induced adaptation.

Recovery following resistance exercise is a complex, multi-faceted process that can involve the combination of a variety of recovery therapies, all with the common goal of restoring physiological and psychological homeostasis. The timing of subsequent exercise bout(s) and the desired outcome from the preceding exercise bout will heavily determine the recovery duration, and whether emphasis is placed upon a single recovery therapy over another. Cold water immersion is a popular post-exercise recovery therapy with extensive use worldwide, largely due to its simplicity, and cost-effective nature of implementation. However, the use of cold water immersion is largely supported only by anecdotal evidence from athletes and practitioners. Little physiological evidence is available as to its mechanisms of action following exercise, both in acute, and long term settings.

There is a paucity of physiological knowledge available regarding post-exercise cold water immersion per se, and this is accompanied with a disparity in the reported physiological and performance responses. The degree of disparity can be attributed not only to the use of cold water immersion protocols differing in e.g. temperature (5 – 15°C), duration (5 – 20 min), immersion depth (individual limb(s) compared with whole-body) and application frequency (single compared with multiple post-exercise immersions), but also to exercise protocols with varied degrees of ecological validity.

In a first experimental study, detailed in chapter 2, I investigated how a bout of active recovery (stationary, low-intensity cycling) compared with a 10 min bout of CWI at 10°C performed after a single high-intensity resistance training session in influencing physiological and performance responses over a 6-hour recovery period. Large physiological responses were observed over the initial period of recovery that could be attributed to a decrease in central (cardiac output) and peripheral (muscle artero-venous) blood flow following cold water immersion. Despite such physiological responses, participants were able to perform more work during a resistance exercise training simulation exercise when cold water immersion was used. Therefore, a second
experimental study, detailed in chapter 3 was undertaken to more closely investigate how performing cold water immersion or active recovery after resistance exercise influence physiological responses over the initial 60 min following the recovery therapy, and how these physiological responses correspond with exercise performance. This study identified marked effects of cold water immersion upon physiological responses such as cardiac output and muscle tissue oxygenation. However, similar to the first study, resistance exercise performance was not impaired following the use of cold water immersion.

These two experimental investigations identified that acutely, a single bout of cold water immersion post-exercise can induce significant and lasting physiological responses. In a chronic environment, such responses could have detrimental effects on adaptation. However, from a performance perspective, resistance exercise performance was maintained, and to some extent improved following cold water immersion. Chronic use of cold water immersion within a training environment may therefore promote greater adaptation, by enhancing recovery between training sessions, and consequently promoting performance within subsequent sessions.

To investigate the effects of regular cold water immersion on adaptation, and the acute cellular and molecular responses to cold water immersion performed after resistance exercise, two further investigations were conducted, which are detailed in chapter 4. These studies involved (1) a 12-week period of resistance training, and (2) an acute bout of resistance exercise, where acute cellular and molecular anabolic responses were assessed from 2-48 hours post-exercise. In both studies, responses were compared between conditions performing either cold water immersion or active recovery post-exercise. The use of cold water immersion significantly reduced strength and hypertrophy adaptation to resistance training, and also augmented the anabolic response.

Taken together, the data generated by these investigations suggest that the acutely, post-exercise cold water immersion may be beneficial in maintaining or enhancing performance undertaken 20 min to 6 hours following resistance exercise, despite marked physiological responses. However, importantly, when cold water immersion is used within a resistance training environment, the accumulation of these acute physiological responses and/or an augmented anabolic response may explain a large inhibition of adaptation that exists. Therefore, the use of cold water immersion as a recovery therapy should be periodised based on the desired outcome of the preceding exercise bout. Cold water immersion may be used when the desired outcome is optimal performance and recovery, however its use should be carefully considered when exercised-induced stress for the promotion of adaptation is desired.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications during candidature

Peer-reviewed Original Investigations


Conference Communications


Roberts LA, Cameron-Smith D, Raastad T, Coombes JS and Peake JM. Cold water immersion reduces strength gains following chronic resistance training. Abstract presented at the Exercise Muscle and Metabolism conference, December 4-6, 2013, Melbourne, Australia.

**Publications included in this thesis**


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<td>Designed the investigation (10%)</td>
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<td>Coombes</td>
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<td>Peake</td>
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Contributions by others to the thesis

Doctors Jonathan Peake, Mark Muthalib and Anthony Shield, along with Professors Ken Nosaka and Truls Raastad contributed to the conception and design of investigations. They also contributed to the critical evaluation and interpretation of the data, along with Prof Jeff Coombes. Professor David Cameron-Smith, Doctor James Markworth and Mr Vandre Cassagrandè Figueiredo facilitated and contributed to the analysis of biological samples from the third and fourth studies.

Statement of parts of the thesis submitted to qualify for the award of another degree

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_Per Angusta Ad Augusta,_
_Mens Sana in Corpore Sano._
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List of Abbreviations

4E-BP1; 4E binding protein 1
ACT; Active recovery
BF; Blood flow
$BF_{\text{macro}}$; Macrovascular blood flow
$BF_{\text{micro}}$; Microvascular blood flow
CIVD; Cold-induced vasodilation
CSA; Cross sectional area
CWI; Cold water immersion
DOMS; Delayed-onset muscle soreness
ERK; Extracellular signal-regulated kinase
GSK3β; Glycogen synthase kinase 3β
IGF-1; Insulin-like growth factor 1
ISOM; Isometric
ISOK; Isokinetic
miRNA; Micro ribonucleic acid
mTOR; Mammalian target of rapamycin
MVC; Maximal voluntary contraction
NIRS; Near infra-red spectroscopy
P13K; Phosphatidylinositol 3-kinase
p70S6K; p70 ribosomal S6 kinase
RNA; Ribonucleic acid
$T_{\text{core}}$; Core temperature
$T_{\text{muscle}}$; Muscle temperature
TPR; Total peripheral resistance
$T_{\text{skin}}$; Skin temperature
VOP; Venous occlusion plethysmography
Chapter 1

*Introduction*
1.1 Exercise recovery and cold water immersion

Resistance exercise, whether competitive- or training-based disturbs a variety of physiological processes within the body, and elicits a movement away from systematic homeostasis. For athletes and the general population alike, there is a delicate balance between restoring homeostasis by optimising recovery, and promoting adaptation. Adaptation is promoted by inducing enough stress to promote supercompensation (Kentta & Hassmen, 1998). Although skeletal muscle is malleable, and can adapt and undergo remodelling in response to a variety of stimuli (Fluck & Hoppeler, 2003), too much stress may lead to an increased risk of injury (Barnett, 2006).

Optimising recovery after resistance exercise often involves a multi-faceted approach, requiring a combination of recovery therapies; such as dietary protein supplementation (Churchward-Venne et al., 2013), compression garments (Kraemer et al., 2010), massage (Zainuddin et al., 2005) or cryotherapy (Tseng et al., 2013). One recovery therapy that has developed overwhelming popularity over the last few years is cold water immersion. The efficacy and physiological basis of cold water immersion has been reviewed extensively elsewhere (Bleakley & Davison, 2010; Bleakley et al., 2012; Leeder et al., 2012).

Despite the popularity of cold water immersion, there is limited physiological evidence for its efficacy. However, the popularity of this therapy may partly be attributed to the perceptive effects of the treatment. Because high-intensity and/or unaccustomed exercise with an eccentric component induces delayed-onset muscle soreness (DOMS) (Clarkson et al., 1986), reducing DOMS is often beneficial for athletes, and is an important focus from a recovery perspective. It is reported that cold water immersion is an effective method of decreasing some of the signs and symptoms of muscle soreness (Leeder et al., 2012; Glasgow et al., 2014), whilst also heightening perceptions of recovery (Cook & Beaven, 2013).

Eccentric exercise can be beneficial for adaptation (Friedmann-Bette et al., 2010), therefore eccentric exercise may be a useful addition to resistance exercise regimes. However, it is unlikely that resistance exercise and resistance training will be compromised solely, or predominantly of eccentric exercise. One major limitation to the current knowledge base regarding post resistance-exercise cold water immersion, is the paucity of investigations utilising traditional resistance exercise. This type of exercise is often comprised of both concentric and eccentric contractions. It is understandable for research investigating the effects of cold water immersion on DOMS to utilise the greatest muscle
damaging exercise preceding the cold water immersion therapy. Such stimuli range from isolated eccentric contractions (Eston & Peters, 1999), maximal and supra-maximal loads (Sellwood et al., 2007; Vaile et al., 2008b), to drop jumps (Howatson et al., 2009) (see Table 1.1).

Performance responses following muscle damaging exercise and cold water immersion are varied, which could be attributed to the severity of the muscle damaging protocols. These factors make it difficult to interpret changes in performance in response to cold water immersion. Following this type of exercise, performance has often been measured using maximal, ‘single-contraction’ exercises, such as single maximal voluntary contractions (MVC’s) of isometric and/or isokinetic torque (see table 1.1 for examples).

Competition or training environments often involve exercises consisting of repeat submaximal concentric and/or eccentric contractions, as opposed to single MVC’s. Therefore it is important to investigate submaximal, ‘repeat-contraction’ exercise alongside MVC’s to identify how cold water immersion may influence performance within these scenarios. To date, no investigation has measured submaximal, ‘repeat-contraction’ exercises performance following resistance training. It is important to address this lack of information. Cold water immersion enhances the perceptions of recovery following exercise (Glasgow et al., 2014), which is associated with subsequent performance (Cook & Beaven, 2013). Consequently, cold water immersion may promote the performance of more ‘repeat-contraction’ work during subsequent exercise bouts. If this hypothesis is correct, it has important implications for performance within both competitive and training environments.

Because DOMS develops over 24–96 h post-exercise (Clarkson et al., 1986), performance following cold water immersion has typically been assessed over a similar timeframe. However, considering that repetitive competitive or training bouts are often performed within 2–12 h of each other, investigating performance responses to traditional resistance exercise over this timeframe is warranted. Furthermore, understanding how performance changes after cold water immersion over such a timeframe may provide insight into how cold water immersion could be used for a recovery in the longer term, where exercise bouts are performed in close proximity to each other repetitively, as within a training program.

Another potential source of variation in responses following post-exercise cold water immersion is the type of cold water immersion protocol that is used. For example,
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<td>Immediately and 24 h post-exercise</td>
<td>Trial day, and 24 and 48 h</td>
<td>Significant change in repetition number and power production over time, decreasing after the first trial. No significant difference in repetition number or power production between conditions</td>
</tr>
<tr>
<td>Jakeman et al. (2009)</td>
<td>Recreationally active females</td>
<td>10 x 10 CMJ with maximal jump height and 90° flexion.</td>
<td>Cold water immersion Control</td>
<td>Single immersion within 10 min post-exercise. To iliac crest for 10 min at 10°C</td>
<td>Pre-exercise and 1, 24, 48, 72 and 96 h post-immersion</td>
<td>Significant time effect for MVC force production. No significant differences existed between conditions.</td>
</tr>
<tr>
<td>Paddon-Jones and Quigley (1997)</td>
<td>Recreationally active males</td>
<td>8 x 8 eccentric elbow flexions at 110% concentric 1-RM</td>
<td>Cold water immersion Control</td>
<td>5 immersions separated by 60 min, starting immediately post-exercise. Full arm 20 min at 5°C</td>
<td>Pre-exercise and 0, 24, 48, 72, 96, 120 and 144 h post-exercise</td>
<td>Significant time effect for isometric and isokinetic MVC force production. No significant differences existed between conditions.</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Protocol Description</td>
<td>Time Considered</td>
<td>Immersion Duration</td>
<td>Findings</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
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<td>---------------------------------------------------------------------------------------</td>
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<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Peiffer et al.</td>
<td>Trained male cyclists</td>
<td>Cycle TTE at ventilatory threshold. Cold water immersion for 5, 10 or 20 min.</td>
<td>25 min post-exercise.</td>
<td>5, 10 or 20 min</td>
<td>Significant decrease in isokinetic torque and peak isometric torque for all conditions, both immediately and 55 min post TTE. No significant differences between conditions.</td>
<td></td>
</tr>
<tr>
<td>Pournot et al.</td>
<td>Trained athletes</td>
<td>2 x 10 min HI circuit of CMJ and 30 sec maximal rowing.</td>
<td>1 and 24 h post-exercise.</td>
<td></td>
<td>Significantly greater CMJ and MVC performance post 1 hr for cold water immersion compared to other modalities.</td>
<td>Significantly greater MVC performance post 24 h for cold water immersion compared to other modalities</td>
</tr>
<tr>
<td>Sellwood et al.</td>
<td>Recreationally active males and females</td>
<td>5 x 10 eccentric leg press. Cold water immersion.</td>
<td>Immediately post-exercise.</td>
<td></td>
<td>No significant change in MVC over time or between conditions</td>
<td></td>
</tr>
<tr>
<td>Vaile et al.</td>
<td>Strength trained males</td>
<td>5 x 10 rep eccentric leg press at 120% 1RM and 10 rep eccentric leg press at 100% 1RM</td>
<td>Immediately, 24, 48, 72 h post-exercise.</td>
<td></td>
<td>Change in squat jump power significant less 48 and 72 h post-exercise for cold water immersion compared to control.</td>
<td>Significantly decreased isometric squat performance for all modalities except for cold water immersion and Contrast at 24, 48 and 72 h post-exercise</td>
</tr>
</tbody>
</table>

MVC, maximal voluntary contraction; TT, time trial; TTE, time to exhaustion; CMJ, counter movement jump; TWI, temperate water immersion; Contrast, contrast water immersion; HWI, hot water immersion; RM, repetition maximum; NMES, neuromuscular electrical stimulation. *Significant difference (p < 0.05).
protocols immersing to the clavicular level, also termed neck-out (Vaile et al., 2008b) versus the exercised limbs only (Sellwood et al., 2007), studies utilising immersion temperatures from 5°C (Sellwood et al., 2007) up to 15°C (Vaile et al., 2008b), and those investigations immersing for durations ranging from 3 min (Sellwood et al., 2007) up to 14 min (Vaile et al., 2008b). See Tables 1-3 for examples of cold water immersion protocols that are commonly used.

Aside from potentially influencing performance, the immersion depth, temperature and duration of cold water immersion will (theoretically) modulate the magnitude of the resulting physiological response (Wilcock et al., 2006). Anecdotally, beliefs of athletes and coaches support the uncertainty regarding what constitutes the optimal cold water immersion protocol. Understanding more regarding the physiological responses and mechanisms of post-exercise cold water immersion in combination with performance measures, will help develop the knowledge base regarding this therapy, and help derive the optimal protocol.

Broadly, the available data on the physiological responses to cold water immersion are limited to thermoregulatory and cardiovascular responses, pertaining to two main avenues of effect. Table 1.2 summarises the studies investigating the thermoregulatory cardiovascular responses to post-exercise cold water immersion. A reduction in tissue temperatures from the water temperature, and hydrostatic pressure from the water immersion may acutely reduce or delay the inflammatory response after exercise by reducing blood flow. This speculation is based on the use of proxy markers of inflammation, such as segmental limb volume and/or circumference changes (Howatson et al., 2009) and systemic cytokine concentrations (Gonzalez et al., 2014). Advocates of cold water immersion associate this proposed reduction and/or delay in post-exercise inflammation with a reduction in DOMS and an increased sense of wellbeing following cold water immersion. Although using proxy measures of inflammation is widespread, no investigations have measured the expression of cytokines and infiltration of inflammatory cells in muscle following cold water immersion in humans.

Inflammation has sometimes been implicated with greater muscle damage (Smith et al., 2008). However, there is a growing body of evidence from animal and human models highlighting a key role for inflammation in promoting adaptive and regenerative responses to muscle injury (Lee et al., 2005; Takagi et al., 2011; Paulsen et al., 2012). The eccentric component of traditional resistance causes micro-damage, localised inflammation and tissue oedema (Clarkson et al., 1986). Very little information is available on regular cold
Table 1.2. Example studies investigating thermoregulatory and vascular responses following exercise and cold water immersion

<table>
<thead>
<tr>
<th>Author</th>
<th>Participants</th>
<th>Exercise</th>
<th>Conditions</th>
<th>Immersion protocol</th>
<th>Timing of measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Broatch et al., In Press)</td>
<td>Recreationally active men</td>
<td>Cycling-based high interval exercise</td>
<td>Cold water immersion TWI TWI placebo</td>
<td>5 min post-exercise. To umbilicus for 15 min at 10°C</td>
<td>During exercise and immersion</td>
<td>Significant reduction in muscle temperature below resting temperature during cold water immersion, which was * below both TWI conditions.</td>
</tr>
<tr>
<td>Ihsan et al. (2013a)</td>
<td>Recreationally active men</td>
<td>30 min running at 70% VO\textsubscript{2} max followed by 10 x 15 sec intervals at 100% VO\textsubscript{2} max</td>
<td>Cold water immersion Control</td>
<td>2 min post-exercise. To gluteal fold for 15 min at 10°C</td>
<td>During exercise and immersion</td>
<td>Significant reduction in skin temperature below resting temperature during cold water immersion, which was * below Control. Total haemoglobin volume was * reduced compared with Control during cold water immersion.</td>
</tr>
<tr>
<td>Ihsan et al. (2014)</td>
<td>Recreationally active men</td>
<td>30 min running at 70% VO\textsubscript{2} max followed by intervals at 100% VO\textsubscript{2} max to exhaustion</td>
<td>Cold water immersion Control</td>
<td>2 min post-exercise. To gluteal fold for 15 min at 10°C</td>
<td>Pre- and post-exercise, post- and 3 h post-immersion</td>
<td>Significant reduction in core temperature following cold water immersion. Significant reduction in muscle temperature following cold water immersion which was * reduced compared with control.</td>
</tr>
<tr>
<td>Mawhinney et al. (2013)</td>
<td>Recreationally active men</td>
<td>Cycling at 70% VO\textsubscript{2} peak until core temperature of 38°C</td>
<td>8°C Cold water immersion 22°C Cold water immersion Control</td>
<td>10 min post-exercise. Immersing of the thighs for 10 min at 8 or 22°C</td>
<td>Pre-exercise, and pre-immersion to 30 min post-immersion</td>
<td>Core temperature remained significantly above baseline following all conditions following the recovery therapy and for 30 min. No * differences existed between conditions. Skin temperature was significantly below baseline following 8 and 22°C immersion and for 30 min post-immersion. Skin temperature was significantly lower following immersion at 8°C. Intramuscular temperature at 1, 2 and 3 cm was significantly reduced at 1 and 30 min following immersion at 8 and 22°C. Temperatures were significantly lower following immersion at 8°C compared with 22°C.</td>
</tr>
<tr>
<td>Peiffer et al. (2009a)</td>
<td>Trained male cyclists</td>
<td>90 min at 80% ventilatory threshold followed by 16km TT</td>
<td>Cold water immersion Control</td>
<td>25 min post-exercise. To mid-sternum for 20 min at 14°C</td>
<td>Pre-exercise, during immersion and to 45 min post-immersion</td>
<td>Femoral vein diameter significantly below resting diameter up to 90 min post-immersion. Core temperature significantly below ACT from 5 to 45 min post recovery therapy, and significantly below resting temperature from 25 to 45 min post-immersion. Skin temperature significantly reduced in response to cold water immersion, and remained significantly below ACT for 45 min following recovery therapies.</td>
</tr>
<tr>
<td>(Peiffer et al., 2009b)</td>
<td>Trained male cyclists</td>
<td>Cycle TTE at ventilatory threshold</td>
<td>Cold water immersion Control</td>
<td>25 min post-exercise. To mid-sternum for 5, 10 or 20 min at 14°C</td>
<td>Pre-post exercise and for 55 min from start of immersion</td>
<td>Significant decrease in core temperature by 50 and 55 min from start of immersion Significant decrease in muscle temperature compared with control, aligned with immersion duration.</td>
</tr>
<tr>
<td>Vaile et al. (2011)</td>
<td>Trained male cyclists</td>
<td>15 min of cycling at 75% peak power followed by 15 min peak work trial</td>
<td>Cold water immersion ACT</td>
<td>5 min post-exercise. ‘Neck out’ for 15 min at 15°C</td>
<td>During exercise bouts, and for 30 min post-immersion</td>
<td>Significant decrease in arm and leg blood flow in response to cold water immersion. Blood flow was significantly reduced following cold water immersion compared with ACT, and remained so for 40 min post-immersion. Significant decrease in core temperature in response to cold water immersion. Core temperature was significantly reduced following cold water immersion compared with ACT, and remained so for 40 min post-immersion.</td>
</tr>
</tbody>
</table>

VO\textsubscript{2}, volume of oxygen consumed; TWI, temperate water immersion; TT, time trial; ACT, active recovery; TTE, time to exhaustion. *Significant difference (\(p < 0.05\)).
water immersion use following resistance exercise. Because inflammation promotes regenerative processes, and cold water immersion reduces inflammation, an important question is posed whether cold water immersion could reduce adaptation.

1.2 Mechanisms of effect and physiological responses

1.2.1 Thermoregulation

Cold water immersion has a pronounced effect on decreasing body temperature, by increasing the heat transfer gradient between the body and the external environment. As exercise duration and/or intensity strongly influence core (T<sub>core</sub>), muscle (T<sub>muscle</sub>) and skin (T<sub>skin</sub>) temperatures, this thermal gradient is enhanced. Exercise performed under higher environmental temperatures can accentuate this gradient even further.

The development of fatigue is the subject of two theories, both of which provide a distinct niche where cold water immersion could be beneficial. Firstly, a ‘critical temperature’ of ~41°C has been proposed (Gonzalez-Alonso et al., 1999). This theory suggests that regardless of T<sub>core</sub> at the beginning of exercise, volitional fatigue will occur at 40.1–40.2°C for T<sub>core</sub>, 40.7–40.9°C for T<sub>muscle</sub>, and 37.0–37.2°C for T<sub>skin</sub>. Secondly, a theory termed the ‘central governor theory’ has been put forward (Noakes, 2012). The theory states that the brain senses progression towards a ‘critical temperature’ limit, and accordingly modulates exercise intensity to prevent the limit being reached. Regardless of the prevailing theory, or the accuracy of either, cold water immersion may play a role in pre-cooling prior to exercise, whether cooling from a resting state or between successive exercise bouts in close proximity to each other. Cooling will reduce T<sub>core</sub>, T<sub>muscle</sub> and T<sub>skin</sub>, allowing a greater ‘thermal reserve’ for metabolic heat production during the following exercise bout. This reserve may potentially allow athletes to exercise for longer periods and/or at a higher intensity prior to the onset of fatigue.

Despite a potential benefit from a thermoregulatory perspective, cooling may not offer the same benefits for muscular performance. It has long been known that muscular contractions have an optimal temperature range (Clarke et al., 1958). Therefore significant reductions in T<sub>muscle</sub>, as reported at rest (Gregson et al., 2011) or following exercise (Peiffer et al., 2009b; Mawhinney et al., 2013) could reduce subsequent muscular performance. The number of investigations reporting thermoregulatory responses to post-exercise cold water immersion, such as T<sub>core</sub>, T<sub>muscle</sub> and T<sub>skin</sub> is sparse (see table 1.2 for details). Consequently, much of the current knowledge about the physiological effects of
Cold water immersion is derived from animal models, or early basic-physiological investigations of humans in a resting state (e.g., Barcroft and Edholm (1943); Clarke et al. (1958).

Cold water immersion may have different effects on maximal and submaximal contractions, due to the varying contributions of the central nervous system in increasing the rate and size of motor unit recruitment for contraction types (Pöyhönen et al., 1999). Therefore, due to the substantial effects of thermoregulatory changes on muscle function and other physiological processes, it is important to quantify the thermoregulatory response to cold water immersion to help understand the dose-response.

Disparity between cold water immersion protocols makes it difficult to compare results between studies. Nevertheless, understanding what magnitude of temperature change is related to a given performance outcome can help understand the dose-response of cold water immersion. A broad spectrum of temperature-dependent physiological responses may be influenced by cold water immersion, however relatively little is known about the biochemical responses (Bleakley & Davison, 2010). Biochemical responses include precursors to the cardiovascular responses, such as nitric oxide and endothelin (see section 1.2.2). Gaining information about other factors relating to haematological responses, such as systemic and oxygenation characteristics (Bacher, 2005) which would play an important role in regulating metabolism (Wheaton & Chandel, 2011) is also important. By understanding more about such responses, a greater knowledge base surrounding the mechanisms of post-exercise cold water immersion can be gathered.
1.2.2 Haemodynamics

Haemodynamic effects on peripheral macrovascular blood flow (arterial) and microvascular (capillary and cutaneous) blood flow or volume have been documented following cold water immersion at rest and post-exercise (Gregson et al., 2011; Mawhinney et al., 2013). A strong, negative relationship has long been known to exist between decreases in $T_{\text{muscle}}$ and blood flow (Barcroft & Edholm, 1943), which exist both during, and up to 60 min following cold water immersion. A paucity of data exist specifically examining peripheral haemodynamics following cold water immersion and resistance exercise. However, as blood flow has been reported to increase in response to any contractile stimulus (Barcroft & Millen, 1939), it is prudent to suggest that the haemodynamic responses during and after cold water immersion would be similar, regardless of the preceding exercise type.

Only three studies within the sport science field have investigated the effects of post-exercise cold water immersion upon peripheral vascular responses (Peiffer et al., 2009b; Vaile et al., 2011; Mawhinney et al., 2013). Whilst another noteworthy study, utilising a resting model, has also been undertaken by Gregson and associates (Gregson et al. 2011). Despite differences in the immersion protocols utilised in these investigations (see table 1.2), significant decreases in blood flow were consistently reported. At rest, immersion in water (regardless of temperature) has been reported to induce marked increases in cardiac output, stroke volume and blood pressure (Bonde-Petersen et al., 1992), however the effects of water immersion on such responses following resistance exercise are unknown. An important consideration to be made is the contribution of hydrostatic pressure compared with temperature on such responses, as hydrostatic pressure has been reported as a key contributor to re-distributing blood flow from the periphery to the thoracic cavity (Wilcock et al., 2006).

Methodological differences in assessing haematological responses between the aforementioned studies make direct comparisons difficult. For example, the use of venous occlusion plethysmography to gauge changes in whole limb perfusion (Vaile et al., 2011), whereas Peiffer et al. (2009b) only measured vascular diameter. Both results however, support the notion of increased total peripheral resistance (TPR) by peripheral vasoconstriction of vessels to redistribute blood supply to other areas. Gregson et al. (2011) were the first to concurrently measure peripheral arterial blood flow cutaneous blood volume, combining Doppler ultrasound with laser Doppler flowmetry. They reported reductions in peripheral arterial blood flow as expected, whilst also observed an increase
in cutaneous microvascular volume. An increase in cutaneous microvascular blood volume may be another compartment where blood flow may be redistributed to following cold water immersion, termed ‘cold-induced vasodilation’. Mawhinney et al. (2013) enhanced the findings of Gregson et al. (2011), combining Doppler ultrasound with laser Doppler flowmetry, but this time after exercise. Similar reductions in peripheral arterial blood flow were reported between both studies were identified, however cold-induced vasodilation did not occur after exercise.

Despite disagreement in identifying cold-induced vasodilation without/after exercise, methodological advances have begun to provide a better understanding of how cold water immersion may influence blood flow to this other compartment. Contrast-enhanced ultrasound is an adapted method displaying promise for quantifying cutaneous microvascular blood flow (Sjoberg et al., 2011). To date however, this method has not been utilised following cold water immersion, having only been adopted following ice application (Selkow et al., 2012). An advantage of this method is the quantification of microvascular flow and volume, as opposed to volume alone, as derived from laser Doppler, or near infrared spectroscopy (NIRS; Ferrari et al., 2011). To date, no investigation has incorporated NIRS to investigate how post-exercise cold water immersion influences microvascular haemodynamics alongside performance responses. Using this approach, a muscle contraction is used to induce reactive hyperaemia (local increase in microvascular blood volume). Magnitude and time characteristics of the NIRS response during the contraction, and during the resulting hyperaemia are subsequently analysed. These characteristics are dependent on intramuscular blood supply, and are reflective of both muscle oxygenation and local metabolism (measured by characteristics of oxy- and de-oxyhaemoglobin). Because cold water immersion influences macrovascular and microvascular blood flow, NIRS can offer a novel insight into the mechanisms by which cold water immersion may influence muscle function. However, a cautious note to make is that NIRS can be influenced by skin blood flow. As cold water immersion may decrease skin blood flow (Gregson et al. 2011), consideration of this needs to be made, alongside maintaining an adequate intramuscular penetration depth for the NIRS signal, to minimise any influence from skin blood flow when utilising this method.

Post-exercise vascular responses are tightly mediated, through the actions of the sympathetic nervous system, and contraction-induced release of potent vasodilator and vasoconstrictor mediators such as ATP (Arciero et al., 2008), nitric oxide (Tschakovsky & Joyner, 2008) and endothelin-1 (Maeda et al., 2002). Important information regarding the
magnitude of blood flow responses to post-exercise cold water immersion have been provided by investigations such as Mawhinney et al. (2013). However, information regarding underpinning biochemical processes and mediators are also warranted. Having information on the biochemical responses to cold water immersion would compliment measures of blood flow or vessel diameters, shed light on underlying mechanisms, and moreover, contribute to the knowledge base relating to the dose-response of cold water immersion in eliciting vascular responses.

As mentioned previously, an important consideration underpinning haemodynamic responses to post-exercise cold water immersion is hydrostatic pressure from the water. Relatively little is known regarding the contribution of hydrostatic pressure to haemodynamic response, despite its involvement in removing metabolites from skeletal muscle (Bleakley & Davison, 2010). Cold water immersion likely induces peripheral blood flow re-distribution through a combination of decreased tissue temperatures resulting from the water temperature, and compression of the immersed limb(s) through hydrostatic pressure from the water. Therefore, a systematic comparison of protocols involving different water temperatures and levels of water immersion is warranted, to differentiate between the effects of water temperature and hydrostatic pressure.

To maximise the effects of cold water immersion, some cold water immersion therapies adopt a neck-out protocol, immersing the body to the clavicular level (e.g., Vaile et al. (2008a) and Vaile et al. (2008b). Equally however, some protocols favour the immersion of only the exercised limb(s) (e.g., Ascensao et al. (2011), Howatson et al. (2009) and Pournot et al. (2011). Hydrostatic pressure has been associated with a reduction in neuromuscular function (Pöyhönen et al., 1999) and the reabsorption of interstitial fluids to decrease oedema (Friden & Lieber, 2001). Therefore, neck-out immersion protocols may maximise these responses. However, as neuromuscular strain and oedema are likely to be limb(s)-specific, it is equally plausible to recommend the immersion of only the exercised limb(s). Once more, a systematic comparison between different immersion depths and body area(s), and resulting responses is required to elucidate the magnitude of which immersion depth influences responses.
1.3 Cold water immersion and responses following resistance exercise

As detailed previously, it is well accepted that cold water immersion results in decreased $T_{\text{core}}$, $T_{\text{muscle}}$, $T_{\text{skin}}$ and oedema. However, much is unknown regarding the magnitude, and time-course of potential physiological interactions that may exist as a result of these responses. Considering the complexities involved in understanding the multiple facets of responses following resistance exercise and cold water immersion, it is understandable that most research to date has focused on exercise-induced muscle damage and DOMS after resistance exercise.

Vigorous and/or unaccustomed exercise, encompassing eccentric muscle contractions elicits exercise-induced muscle damage and DOMS, often measured by marked reductions in voluntary muscle function and/or biochemical markers such as creatine kinase over 24–96 h following exercise (Clarkson et al., 1986; Byrne et al., 2004). In addition, muscle soreness resulting from muscle damage is associated with reports of decreased perceptions of recovery, and increases in muscular inflammation, sensitivity and pain (Miles & Clarkson, 1994).

Although eccentric-only resistance exercise can play a useful role within regular resistance training (Friedmann-Bette et al., 2010), more traditional resistance training requires a combination of both concentric and eccentric contractions. Because the contribution of eccentric contractions to traditional resistance exercise is lower than eccentric-only exercise, exercise-induced muscle damage and DOMS are typically less severe after resistance exercise. Nevertheless, cold water immersion is often used following resistance exercise with the intention of reducing the severity of some of the symptoms of exercise-induced muscle damage (Glasgow et al., 2014), and has been reported to be a successful method of accomplishing this (Bleakley et al., 2012; Leeder et al., 2012).

Applications of ice to animal models of skeletal muscle damage (crush injury) have provided insights into how adaptive and regenerative processes may respond to post-resistance exercise cold water immersion. Some considerations are warranted when extrapolating results from this model to post-resistance exercise cold water immersion, including (i) ice application versus cold water immersion, and (ii) muscle crush injury versus exercise-induced muscle damage. Nevertheless, ice application does result in similar reductions in $T_{\text{skin}}$ and $T_{\text{muscle}}$ (Dykstra et al., 2009) compared with cold water immersion.
immersion, and also reduces blood flow (Wilson et al., 2007; Topp et al., 2011), despite the absence of hydrostatic pressure that accompanies cold water immersion. Furthermore, eccentric contractions are known to cause ultrastructural damage consistent with that induced by crush injury (Newham et al., 1983; Armstrong, 1990; Grounds et al., 1992). Key studies adopting this crush model include those by Lee et al. (2005) and Takagi et al. (2011). Both investigations reported an attenuation of the inflammatory response following ice application, notably by reductions in local and circulating leukocytes (Lee et al., 2005), and intramuscular macrophage infiltration, and insulin like growth factor-1 (IGF-1) and transforming growth factor-β1 expression (Takagi et al., 2011). Interestingly, Takagi et al. (2011) also reported that icing after crush injury significantly delayed muscle regeneration at 14 and 28 days. These responses included an increase in fibres expressing centrally located nuclei at 14 days, a decreased fibre cross sectional area (CSA) at 28 days, and increased collagen deposition at 14 and 28 days following ice application. These indices suggest that cold application may be detrimental for promoting adaptation and regeneration following muscle damage. These findings are further supported by a growing body of evidence supporting a role for acute inflammation in the promotion of muscle regeneration and repair (Paulsen et al. (2012).

Despite the role for inflammation in promoting muscle adaptation, other important mechanisms include the biochemical processes of overlapping pathways that underpin protein synthesis. These pathways are highly sensitive to the mechanical stimuli of exercise. These pathways involve the phosphorylation of key kinases and post-transcriptional modification of certain genes. Key factors that regulate muscle protein synthesis include the IGF-1, Akt-mammalian target of rapamycin (mTOR), and phosphatidylinositol 3-kinase (P13K)-Akt-glycogen synthase kinase 3β (GSK3β) pathways (Glass, 2003; Hornberger & Chien, 2006). The Akt-mTOR pathway has received most attention with regard to post-exercise protein synthesis (Drummond et al., 2009), with particular recognition of the role of downstream proteins such as p70S6K and 4E-BP1 in promoting adaptation in response to exercise and/or amino acid stimuli (Bodine et al., 2001) (see figure 1.1). In addition, skeletal muscle stem cells (satellite cells) have been reported to play an important role for both muscle regeneration and hypertrophy (Blaauw & Reggiani, 2014). These cells exist in a quiescent state between the sarcolemma and basal lamina, where they are activated by an exercise stimulus (Schiaffino et al., 1972, 1976) and used to support hypertrophy of the cell (Bruusgaard et al., 2010). The activity of proteins involved in these regulatory pathways may well depend on tissue temperature, and follow a ‘U-shaped’ pattern (Jaenicke, 1990). Manipulation of temperature is known to
alter the activity of important processes such as protein turnover (Baracos et al., 1984) and cell membrane permeability (Goodford, 1971) which would have implications for the utility of cold water immersion after exercise. No investigation to date has utilised molecular techniques to investigate molecular and proteomic responses after cold water immersion \textit{per se}, or following post-exercise cold water immersion where the activity of these responses is elevated.

As seen in Table 1, investigations exploring responses to post-resistance exercise cold water immersion have utilised exercise-induced muscle damage protocols as opposed to more traditional resistance exercise. Therefore, it is important to understand how cold water immersion might influence the molecular mechanisms involved in the adaptation to traditional resistance exercise. This information will improve our understanding of how cold water immersion can influence recovery in the short-term, and also how any short-term responses may manifest as long-term training adaptations.

A paradox exists in relation to the use of cold water immersion within a training environment. If cold water immersion can promote the recovery of performance and wellbeing following a resistance training session, this could increase the quality and quantity of work performed within subsequent sessions. Therefore, over the long-term, a chronic repetition of this may lead to enhanced adaptation. Conversely, however, considering the potential for cold water immersion to attenuate both inflammation and

\textbf{Figure 1.1} Graphical representation of the predominant Akt-mTOR pathway kinases (blue ovals), signalling relationships (arrows) and phosphorylation (red circles).
protein synthesis in the acute phase following resistance exercise, it is plausible to postulate that this may in fact inhibit training-induced adaptation.

Only two investigations have investigated the use of cold water immersion within a resistance training environment exceeding a 1 week period (see table 1.3), namely Yamane et al. (2006) and more recently, Fröhlich et al. (2014). Limitations exist regarding the practicality of the research designs in both these investigations. Yamane et al. (2006) used a resistance training protocol that only involved handgrip exercise, and also extrapolated performance results (1-RM) of this small muscle group to whole-body performance. Similarly, Fröhlich et al. (2014) only trained the hamstring muscle group, and they only reported for changes in 1-RM and 12-RM strength. Despite these limitations, both studies concluded that regular cold water immersion attenuated training-induced adaptations. In order to investigate this paradox, comprehensive investigations utilising more realistic resistance training protocols, in combination with sensitive measurements of muscle mass and performance are warranted. This approach would provide more comprehensive insights into the effects of cold water immersion on functional, morphological and molecular adaptations to resistance training.

In summary, balancing recovery and adaptation following resistance exercise is a complex process, involving a number of underlying and overlapping physiological processes. Understanding physiological, mechanistic and performance responses to acute and chronic use of cold water immersion is imperative. A better understanding of the physiological responses is needed to enhance our understanding of the mechanisms of effect. Knowledge of these mechanisms will provide dose-response information, and help form an optimal immersion protocol. When this dose response information is combined with performance indicators, it will contribute to the formation of best-practice guidelines on when cold water immersion should be used, with regards to recovery or adaptation.

This knowledge is important, considering the popularity and widespread use of cold water immersion. If cold water immersion induces negative adaptive responses following exercise, incorrect use could have significant implications. For example, during pre-season or within a training macrocycle, muscle adaptation may be the primary aim of training. Using cold water immersion within these environments may then be detrimental. On the other hand, following training session or competitive bouts in-season where optimising recovery is the main aim, cold water immersion could be a viable and effective recovery therapy.
Table 1.3. Summary of studies investigating adaptation to strength training with regular cold water immersion use

<table>
<thead>
<tr>
<th>Author</th>
<th>Participants</th>
<th>Exercise</th>
<th>Conditions</th>
<th>Immersion protocol</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burke et al. (2000)</td>
<td>Recreationally active males and females</td>
<td>5 consecutive days of hip extensor exercise</td>
<td>Cold water immersion HWI Control</td>
<td>Immediately after each training session. To gluteal fold for 10 min at 8°C Immediately after each training session. To iliac crest for 3 x 4 min at 12°C, each separated by 30 sec</td>
<td>Hip extensor isometric strength</td>
<td>Significant increase in isometric strength in all conditions post-training. Significantly greater increase in isometric strength following cold water immersion compared with other conditions.</td>
</tr>
<tr>
<td>Fröhlich et al. (2014)</td>
<td>Strength trained males</td>
<td>Knee curl exercise 2 d·wk⁻¹ for 5 weeks.</td>
<td>Cold water immersion Control</td>
<td></td>
<td>Hamstring 1-RM and 12-RM strength</td>
<td>Significant increase in 1-RM over time in both conditions, * less increase following cold water immersion. Significant increase in 12-RM over time in both conditions, * less increase following cold water immersion.</td>
</tr>
<tr>
<td>Yamane et al. (2006)</td>
<td>Recreationally active males</td>
<td>Handgrip exercise 3 d·wk⁻¹ for 4 weeks.</td>
<td>Cold water immersion Control</td>
<td>Within 3 min after each training session. Forearm for 20 min at 10°C</td>
<td>Handgrip strength endurance and brachial artery diameter</td>
<td>Significant increase in muscular endurance in both conditions, no difference between conditions. Significant increase in brachial artery diameter in Control condition, no * increase following cold water immersion.</td>
</tr>
</tbody>
</table>

HWI, hot water immersion; RM, repetition maximum. *Significant difference (p < 0.05).
1.4 Aims

The overall objective of this thesis was to examine physiological and biochemical responses to cold water immersion after resistance exercise, and how they influence subsequent performance. This aim was achieved through four distinct original investigations.

The primary aim of the first investigation was to investigate how cold water immersion following high intensity bi-lateral resistance exercise impacts the recovery of maximal and sub-maximal muscle function. Limitations of the current knowledge base is that (i) performance responses to cold water immersion have only utilised MVC’s to measure performance, and (ii) responses have been investigated in response to exercise-induced muscle damage and not more traditional resistance exercise. Little information is known about how cold water immersion influences sub-maximal performance, even though sub-maximal performance is a component of both competitive exercise bouts and exercise training. A secondary aim was to extend the existing knowledge on the physiological responses resulting from post-exercise cold water immersion. Although it is known that cold water immersion reduces peripheral macrovascular blood flow, much less information is known on physiological responses that accompany this response. In this first study, changes in maximal and submaximal muscle function, venous blood gases, endothelin-1, myoglobin, lactate and interleukin-6 (IL-6) were measured over a period of 6 hours following resistance exercise and cold water immersion or active recovery.

The primary aim of the second investigation was to examine the effects of cold water immersion on central and peripheral haemodynamics during the early phase of recovery after resistance exercise. Peripheral macrovascular (arterial) and microvascular (capillary and cutaneous) responses ≤ 60 min of following endurance and interval based exercise and cold water immersion have previously been documented (Mawhinney et al., 2013). However, a paucity of information exists regarding central (cardiac output, stroke volume and parasympathetic activity) and peripheral (microvascular blood volume) haemodynamics over this time frame. These measures were implemented within a tightly regulated unilateral exercise model. This model allowed resistance exercise to be used as a central and peripheral hyperaemic stimulus. Furthermore, this model allowed the measurement of peripheral hyperaemic responses, together with maximal and sub-maximal performance.

The aim of the third study was to investigate how regular use of cold water immersion following resistance exercise influences adaptation within a training
environment. Only two previous investigations have investigated the efficacy of cold water immersion within a strength training environment (Yamane et al., 2006; Fröhlich et al., 2014). However, both investigations were subject to limitations in their design. The third investigation sought to address these limitations by (i) implementing a bi-lateral lower body exercise model, (ii) encompassing exercises that are more representative of an athletic resistance training program (iii) implementing a 12-week training intervention to maximise the training-induced effects, and (iv) adopting extensive, and sensitive assessments of responses such as isokinetic dynamometry, MRI and muscle biopsy analysis of muscle fibre architectural responses.

The final study in this thesis aimed to explore the cellular and molecular responses occurring in muscle 2–48 h following resistance exercise and cold water immersion. To date, limited information on cellular and molecular responses in muscle following post-exercise cold water immersion. The only information available has come from three investigations, having examined glycogen re-synthesis (Tucker et al., 2012; Gregson et al., 2013) and angiogenic and mitochondrial mRNA responses (Ihsan et al., 2014) after cold water immersion. No information is available regarding cellular and molecular responses to resistance exercise. This information is required in order to provide a better understand or the acute physiological mechanisms that are influenced by cold water immersion, and how these acute responses may underpin chronic adaptation to training.
Chapter 2

Title: Cold water immersion enhances recovery of submaximal muscle function following resistance exercise

Authors: Roberts LA, Nosaka K, Coombes JS, Peake JM.

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Cold water immersion enhances recovery of submaximal muscle function after resistance exercise

Llion A. Roberts,1,2 Kazunori Nosaka,3 Jeff S. Coombes,1 and Jonathan M. Peake2,4

1School of Human Movement Studies, The University of Queensland, Brisbane, Queensland, Australia; 2Centre of Excellence for Applied Sport Science Research, Queensland Academy of Sport, Brisbane, Queensland, Australia; 3School of Exercise and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia; and 4School of Biomedical Sciences and Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia

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Cold water immersion enhances recovery of submaximal muscle function after resistance exercise. Am J Physiol Regul Integr Comp Physiol 307: R998–R1008, 2014. First published August 13, 2014; doi:10.1152/ajpregu.00180.2014.—We investigated the effect of cold water immersion (CWI) on the recovery of muscle function and physiological responses after high-intensity resistance exercise. Using a randomized, cross-over design, 10 physically active men performed high-intensity resistance exercise followed by one of two recovery interventions: 1) 10 min of CWI at 10°C or 2) 10 min of active recovery (low-intensity cycling). After the recovery interventions, maximal muscle function was assessed after 2 and 4 h by measuring jump height and isometric squat strength. Submaximal muscle function was assessed after 6 h by measuring the average load lifted during 6 sets of 10 squats at 80% of 1 repetition maximum. Intramuscular temperature (1 cm) was also recorded, and venous blood samples were analyzed for markers of metabolism, vasoconstriction, and muscle damage. CWI did not enhance recovery of maximal muscle function. However, during the final three sets of the submaximal muscle function test, participants lifted a greater load (P < 0.05, Cohen’s effect size: 1.3, 38%) after CWI compared with active recovery. During CWI, muscle temperature decreased ~7°C below postexercise values and remained below preexercise values for another 35 min. Venous blood O2 saturation decreased below preexercise values for 1.5 h after CWI. Serum endothelin-1 concentration did not change after CWI, whereas it decreased after active recovery. Plasma myoglobin concentration was lower, whereas plasma IL-6 concentration was higher after CWI compared with active recovery. These results suggest that CWI after resistance exercise allows athletes to complete more work during subsequent training sessions, which could enhance long-term training adaptations.

cryotherapy; recovery; performance; thermoregulation; muscle damage; blood gases

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Address for reprint requests and other correspondence: J. M. Peake, Institute of Health and Biomedical Innovation, Kelvin Grove, QLD 4059, Australia (e-mail: jonathan.peake@qut.edu.au).
in muscle O$_2$ saturation after cold water immersion are also associated with changes in venous blood O$_2$ saturation. This information is important because O$_2$ availability regulates motor unit activity in skeletal muscle (35), which might have implications for exercise performance. Cold water immersion has been proposed to benefit recovery from exercise by reducing inflammation (3). However, research into the effects of cryotherapy on systemic markers of inflammation (e.g., cytokines and C-reactive protein) after eccentric exercise (16, 39, 42, 45) and more traditional resistance exercise (10, 12, 13, 23) has produced inconsistent findings. These studies generally collected blood samples immediately, 1 h, or 24 h after exercise and may therefore have overlooked peaks in the inflammatory response that occur between 1 and 24 h after exercise (38). Further research is needed to understand the effects of cold water immersion on inflammation during the first few hours after resistance exercise.

The primary aim of the present study was to compare the effects of cold water immersion versus active recovery on short-term restoration of maximal and submaximal muscle function after resistance exercise. A secondary aim of the present study was to extend existing knowledge of the physiological mechanisms by which cold water immersion could enhance recovery from exercise. To do this, we measured muscle temperature during and after cold water immersion. We also assessed muscle soreness and swelling and collected blood samples at regular intervals in the first few hours after cold water immersion to measure changes in venous blood O$_2$ saturation, ET-1, and IL-6 as systemic markers of muscle metabolism and inflammation. We hypothesized that compared with active recovery, cold water immersion would 1) enhance recovery of maximal and submaximal muscle function; 2) increase serum ET-1 concentration; and 3) reduce muscle temperature, muscle soreness, and swelling, venous blood O$_2$ saturation, and plasma myoglobin and IL-6 concentrations.

**METHODS**

**Subjects.** Ten physically active young men (means ± SD, age: 21.3 ± 1.6 yr, height: 1.81 ± 0.06 m, and body mass: 84.7 ± 12.4 kg) volunteered to participate in this study. All participants were strength training 2–3 times/wk at the time of the study but were not accustomed to cold water immersion. The experimental procedures and potential risks were explained to the participants before they provided written informed consent. All participants were screened for contraindications to resistance exercise. This study was approved by the Human Research Ethics Committee of the University of Queensland.

**Study design.** Participants first completed two familiarization sessions to introduce them to the exercise protocols that would be used in the experimental trials followed by baseline testing to measure their strength. Two weeks after the familiarization sessions and baseline testing, participants completed two experimental trials separated by 14 days. They returned to the lab to replicate the baseline testing and completed a training record covering this period. Training was replicated for the period between the first and second experimental trial. The order of the experimental trials was randomized and counterbalanced. These trials involved a single, standardized bout of high-intensity resistance exercise followed by cold water immersion or active recovery. Participants refrain from consuming alcohol, caffeine, and tobacco over the 24 h preceding both experimental trials. They also refrain from consuming any nutritional supplements between the baseline testing and completion of their second experimental trial. They completed a 24-h nutritional record before the first experimental trial, which was photocopied and returned to them to replicate for the 24 h before the second trial. They refrained from participating in any lower body exercise training for 48 h before each experimental trial.

**Preexercise sessions.** Preexperimental sessions were completed 14 days before the first experimental trial and included a repetition maximum (RM) testing session, two familiarization sessions, and a baseline testing session. All preexperimental sessions and experimental trials were performed within the same temperature–controlled laboratory (temperature: 24.3 ± 0.6°C and humidity 48.6 ± 1.2%). On the first day, RM testing and the first familiarization session were performed. Participants were tested to determine their RM for back squats, front squats, and incline leg presses. These RM data were used to set the workload for resistance exercise in the experimental trials. After 5–10 min of stationary cycling, participants performed a first repetition at an estimated 90% of RM for each exercise. If successful, participants repeated each exercise with gradually increasing resistance until failure or the loss of correct technique (as assessed by L. A. Roberts). A minimum of 3 min of recovery was allowed between attempts. A successful RM was recorded as the greatest mass lifted with correct form through a complete range of movement. Approximately 15–30 min later, participants started the first familiarization session. The familiarization session involved participants practicing the maximal muscle function tests that would be used during the experimental trials. On the second day, participants repeated the familiarization session from the first day before they practiced the submaximal muscle function test. More details of these tests are described below. On the third day, participants were tested to determine their baseline maximal and submaximal muscle function. After these baseline measures were recorded, participants were familiarized with the cold water immersion therapy to be used during the experimental trials.

**Experimental trials.** For each trial, the participants arrived at the laboratory at 8:30 AM, having eaten breakfast at 7 AM, and consumed 10 ml/kg water over the preceding 2 h. Resting venous blood samples were collected upon arrival. Maximal and submaximal muscle functions were not measured on the morning of each experimental trial due to time constraints and also to avoid any residual fatigue from the testing procedures that could affect performance during the subsequent resistance training session. Instead, maximal and submaximal muscle functions measured during the third day of the preexperimental sessions were used to compare with changes in maximal and submaximal muscle functions measured during each trial (see details above).

After blood samples had been collected, resting superficial muscle temperature (1 cm deep) was recorded, and segmental limb volumes were calculated (as described below). Participants then completed a high-intensity resistance training session lasting ~1 h. This session consisted of the following: 6 sets of front and back squats at loads corresponding to 8, 8, 10, 12, 10, and 10 RM; 3 sets of 12 walking dumbbell lunges with a total mass corresponding to 40% of body mass; and 3 sets of 12 countermovement drop jumps from a height of 50 cm. Recovery time between sets was 90 s, with 120 s between exercises. Strong verbal encouragement was provided where required to maintain repetition tempo, form, and recovery periods. Participants drank water ad libitum during the training session. A venous blood sample was collected, and muscle temperature, upper leg circumference, muscle soreness, and maximal muscle function were measured as soon as possible after the training session.

In the first 15 min after resistance exercise, participants changed clothes and moved to a different room, where they started a 10-min recovery intervention consisting of cold water immersion or active recovery. After participants completed these recovery interventions, they recovered for a period of 6 h. Maximal muscle function was

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assessed again at 2 and 4 h, whereas submaximal muscle function was measured 6 h after completion of the recovery interventions.

During the 6-h recovery period (i.e., after cold water immersion or active recovery), participants remained in the laboratory (23–25°C) and were allowed to walk around at a low intensity. During the first 4 h of recovery, they were given standardized meals that provided 1.2 g·kg⁻¹·h⁻¹ carbohydrate and 0.4 g·kg⁻¹·h⁻¹ protein.

Cold water immersion and active recovery. For the cold water recovery intervention, participants sat in an inflatable bath (iBody, iCool Australia, Miami, QLD, Australia) containing water at 10 ± 0.3°C. Participants immersed their body up to their clavicle continuously for 10 min in water. We chose 10°C as a suitable water temperature because this has commonly been used in a previous cold water immersion study (2). Furthermore, recent studies have indicated that 10–15 min of immersion in water at 8–10°C is effective for reducing tissue temperature, blood flow, microvascular blood volume, and metabolic activity in skeletal muscle after exercise (4, 21, 22, 31).

For the active recovery intervention, participants exercised on a cycle ergometer (Wattbike, Wattbike, Nottingham, UK) for 10 min at a low, self-selected intensity. We selected active recovery as a control condition or comparison for cold water immersion instead of passive recovery because, in reality, it is unlikely that athletes would do no activity at all while recovering from prior exercise. Participants cycled a mean ± SD distance of 3.62 ± 0.25 km at an average power output of 45 ± 7 W.

Maximal muscle function tests. Participants completed four tests of maximal muscle function, including a countermovement jump, an unweighted squat jump, a weighted squat jump, and an isometric squat. They performed these tests in random order at 2 and 4 h after the recovery interventions.

Countermovement jump performance was measured while participants adopted a stance shoulder-width apart, holding a wooden bar in the conventional position for a back squat. On instruction, participants maximally flexed their knees before jumping vertically for maximum height. Their hands remained in contact with the wooden bar at all times while the wooden bar remained in contact with the upper back. They performed three jumps, and data from the best jump were analyzed. Participants performed unweighted and weighted squat jumps while holding a wooden bar (unweighted, ~500 g) or a barbell (Australian Barbell) loaded to 30% of RM (weighted, 37 ± 9.8 kg) in the same position. They performed three maximal jumps, and the data from the best jump were recorded. For both jumps, participants lowered their body to 90° knee flexion and paused for 2 s before jumping for maximum height.

Jump performance was measured using a portable force transducer (GymAware, Kinetic Performance Technology). Data were transmitted from the transducer to a hand-held personal digital assistant. For the countermovement jump and unweighted squat, data were calculated for jump height, work, mean velocity, and peak velocity. For the weighted squat, mean and peak power were also calculated. These calculations were done using native GymAware software (Kinetic Performance Technology). The coefficient of variation for these tests was established during the baseline testing procedures. It was 1.9% for countermovement jump height, 2.8% for unweighted squat jump height, and 2.9% for weighted squat jump height.

Isometric squat strength was measured using a modified back squat. Participants adopted a standard squat position on a Smith machine at one-third of maximum depth and pressed upward against a stationary bar (25, 44). Participants positioned themselves on a force platform (Kistler, Ostfildern, Germany) while ground reaction force data were collected at 1,000 Hz using native software (BioWare version 5.1.3). Participants were instructed to press upward against the bar as quickly and as forcefully as possible without any prior flexion of the knees, spine, or hips. They performed three efforts lasting 3 s each, and data from the best effort were analyzed. Data were filtered with a Butterworth fourth-order digital low-pass filter with a cutoff frequency of 10 Hz before analysis. Isometric squat characteristics comprised peak vertical force and force development. The rate of force development was calculated over 30, 50, 100, and 200 ms. The coefficient of variation for maximum isometric strength was established as 0.9% during the baseline testing procedures.

Submaximal muscle function test. The submaximal muscle function test was similar to that previously described (25). This test was designed to simulate a second training session on the same day (i.e., after the initial resistance training session). It consisted of six sets of back squat repetitions performed at 80% of RM separated by 3 min of passive recovery. Participants attempted to complete 10 repetitions/set. If they were unable to complete 10 repetitions during any set, they stopped lifting and rested for 3 min before beginning the next set. Participants continued this sequence until they had attempted to complete six sets. Repetitions were performed using a squat rack (Force Fitness Equipment, Baltimore, MD), and the number of successful repetitions and mass lifted per set were recorded in addition to the total mass lifted over the six sets. The coefficient of variation for the total mass lifted during this high-intensity resistance exercise test was established as 0.7% during the baseline testing procedures.

Muscle temperature measurement. Muscle temperature was recorded before and after the training session, continuously throughout the recovery interventions, and over the following 2 h. It was measured by inserting an 18-gauge cannula into the vastus lateralis muscle to a depth of 1 cm. Thigh skinfold thickness was measured using Harpenden skinfold calipers (HSK BI, Baty, West Sussex, UK) and divided by two to determine subcutaneous fat thickness (19). A fine wire thermistor (T204E, Physitemp Instruments) was inserted through the cannula to the required depth and removed once temperature had stabilized (~5 s, pre- and postexercise). In a similar manner, a cannula was inserted before the recovery intervention and secured with medical tape and waterproof dressing. Data were logged at 1 Hz using a portable logger (SQ2020, Grant Instruments) and averaged over 1-min intervals for the recovery intervention and 5-min intervals for the first 2 h of recovery.

Blood collection and analysis. Venous blood samples were collected into vacuum tubes containing serum, EDTA, and lithium heparin. Serum vacutainers were left to clot at room temperature for 30 min before centrifugation. Vacutainers containing EDTA and lithium heparin were immediately put on ice and then centrifuged at 1,000 g at 4°C for 10 min. Plasma and serum were aliquotted and stored at −80°C for later analysis. Samples were collected before and after the initial resistance exercise and 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 4 h, and 6 h after the recovery intervention. A portion (100 μL) of fresh heparinized blood from the samples at all time points except 4 and 6 h after the recovery intervention was pipetted into C4+ cartridges (Abbott Point of Care). These cartridges were then immediately inserted into a portable point-of-care device (iSTAT, Abbott Point of Care) to measure blood lactate concentration, pH, venous blood O₂ saturation, and venous blood CO₂ saturation.

Plasma myoglobin concentration was measured using an immunoassay (Roche Diagnostics) and an automated clinical analyzer (Cobas E411, Roche Diagnostics). Plasma IL-6 concentration was measured by ELISA (Quantikine HS ELISA, R&D Systems, Minneapolis, MN). Serum ET-1 concentration was also measured by ELISA (Quantikine ELISA, R&D Systems). Myoglobin, IL-6, and ET-1 were analyzed in duplicate, with sample means taken as the result. The intra-assay coefficient of variation was 1.7% for myoglobin, 9.3% for IL-6, and 6.0% for ET-1.

Limb volume assessment. Segmental limb volume for the lower and upper thigh of the right leg was calculated based on three circumferences. Anthropometric tape was used to measure the circumference 1) above the knee, 2) at mid thigh, and 3) at the subgaleal fold. Positions were marked with a permanent marker for site identification during
and between trials. Limb volume between circumferences 1 and 2 (lower thigh) and between circumferences 2 and 3 (upper thigh) were calculated based on the formula proposed by Katch and Katch (15). The coefficient of variation for upper and lower limb volume assessment was 0.5 and 0.6%, respectively.

Muscle soreness perception. Leg muscle soreness was assessed under two conditions: 1) standing with feet shoulder-width apart and 2) squatting to a 90° knee angle, so that the quadriceps muscles were under tension. Perceived soreness was rated on a horizontal visual analog scale from 0 (no soreness) to 100 (maximal soreness).

Statistical analysis. Statistical analysis was conducted using the Statistical Package for Social Sciences program (version 19, IBM, New York, NY). With the exception of IL-6, all data were normally distributed (as confirmed using the Shapiro-Wilks test) and were analyzed using two-factor repeated-measures ANOVA. When significant trial and time × trial interaction effects were evident (P < 0.05), paired t-tests were used to compare changes over time and between trials. IL-6 data were analyzed using Friedman’s two-way ANOVA by ranks and a Wilcoxon’s signed rank test. The false discovery rate was used for multiple comparisons of time points within and between trials. Absolute values for intramuscular temperature, blood gases, pH, and ET-1 that were recorded preexercise, 15 min, 30 min, and 1 h postexercise were pooled. Cohen’s effect size (d) was calculated to compare the magnitude of changes over time and differences between the trials and assessed as follows: 0.2 = small effect, 0.5 = moderate effect, and 0.8 = large effect. All data except IL-6 are presented as means ± SD; IL-6 data are presented as means ± interquartile range. Significance was set at a level of P < 0.05.

RESULTS

Maximal muscle function. Performance in all jumps decreased after the resistance exercise (P < 0.05) but then progressively increased from 2 to 4 h after resistance exercise in both cold water immersion and active recovery trials (Table 1). Countermovement and unweighted squat jump

<table>
<thead>
<tr>
<th>Time</th>
<th>Countermovement jump</th>
<th>Unweighted squat jump</th>
<th>Weighted squat jump</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before exercise</td>
<td>Immediately after exercise</td>
<td>2 h postexercise</td>
</tr>
<tr>
<td>Jump height, cm</td>
<td>CWI 33.7 ± 6.4</td>
<td>26.7 ± 8.2*</td>
<td>29.6 ± 7.1*</td>
</tr>
<tr>
<td></td>
<td>ACT 27.6 ± 6.0*</td>
<td>30.6 ± 6.9</td>
<td>30.6 ± 6.3</td>
</tr>
<tr>
<td>Peak velocity, m/s</td>
<td>CWI 3.4 ± 0.5</td>
<td>3.1 ± 0.5*</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>ACT 3.1 ± 0.5*</td>
<td>3.3 ± 0.3</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Mean velocity, m/s</td>
<td>CWI 2.0 ± 0.4</td>
<td>1.7 ± 0.2*</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>ACT 1.7 ± 0.1*</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Work, kJ</td>
<td>CWI 39.6 ± 8.7</td>
<td>32.1 ± 8.9*</td>
<td>35.0 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>ACT 32.8 ± 6.9*</td>
<td>36.5 ± 7.6</td>
<td>35.3 ± 8.5</td>
</tr>
<tr>
<td>Jump height, cm</td>
<td>CWI 31.3 ± 6.7</td>
<td>24.9 ± 7.4*</td>
<td>27.0 ± 6.1*</td>
</tr>
<tr>
<td></td>
<td>ACT 23.7 ± 4.7*</td>
<td>28.3 ± 6.1*†</td>
<td>27.6 ± 6.6*</td>
</tr>
<tr>
<td>Peak velocity, m/s</td>
<td>CWI 3.1 ± 0.4</td>
<td>2.8 ± 0.3*</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>ACT 2.9 ± 0.3*</td>
<td>3.0 ± 0.3</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>Mean velocity, m/s</td>
<td>CWI 1.6 ± 0.2</td>
<td>1.5 ± 0.2*</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>ACT 1.5 ± 0.1*</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Jump height, cm</td>
<td>CWI 35.7 ± 8.5</td>
<td>28.9 ± 8.0*</td>
<td>34.5 ± 10.9</td>
</tr>
<tr>
<td></td>
<td>ACT 30.1 ± 5.3</td>
<td>34.3 ± 8.3</td>
<td>34.6 ± 6.4</td>
</tr>
<tr>
<td>Weighted squat jump</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jump height, cm</td>
<td>CWI 16.3 ± 3.4</td>
<td>12.5 ± 3.1*</td>
<td>13.7 ± 3.1*</td>
</tr>
<tr>
<td></td>
<td>ACT 13.4 ± 3.6*</td>
<td>14.6 ± 3.5</td>
<td>14.6 ± 3.4</td>
</tr>
<tr>
<td>Peak velocity, m/s</td>
<td>CWI 2.4 ± 0.2</td>
<td>2.1 ± 0.2*</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>ACT 2.1 ± 0.3*</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>Mean velocity, m/s</td>
<td>CWI 1.2 ± 0.1</td>
<td>1.0 ± 0.1*</td>
<td>1.1 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>ACT 1.1 ± 0.1*</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Peak power, kW</td>
<td>CWI 1.3 ± 0.4</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>ACT 1.1 ± 0.4*</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Mean power, W</td>
<td>CWI 503 ± 188</td>
<td>436 ± 137*</td>
<td>464 ± 156*</td>
</tr>
<tr>
<td></td>
<td>ACT 443 ± 117*</td>
<td>454 ± 131*</td>
<td>453 ± 136*</td>
</tr>
</tbody>
</table>

Data are means ± SD. CWI, cold water immersion trial; ACT, active recovery trial. Baseline strength and power data were recorded during the third day of preexperimental sessions. See Experimental trials in METHODS for details. *Significant difference from preexercise (P < 0.05); †significant difference between trials (P < 0.05).
After active recovery, for some inexplicable reason, muscle temperature was 3–4°C below postexercise values at 2 h and 4 h (approximately −3.5 cm or −11% for both countermovement and unweighted squat jump height). Performance in all jumps was generally similar after cold water immersion and active recovery. Similar trends existed for changes in peak velocity, mean velocity, work, peak power, and mean power during the recovery period for all jumps. Peak isometric strength followed a similar trend, decreasing postexercise (P < 0.05) and recovering toward preexercise values by 4 h (Table 2). There were no significant differences in the rate of force development between the trials at any time point (Table 2).

**Submaximal muscle function.** Submaximal muscle function was assessed at 6 h after the recovery interventions by measuring the average and total load that participants lifted during 6 sets of 10 squats at 80% of one RM. There were no significant differences in average or total load between cold water immersion and active recovery trials during the first three sets of the submaximal function test (P > 0.05; Fig. 1). However, the average load (P = 0.025, d: +1.3, +38%) and total load (P = 0.021, d: +0.7, +16%) that participants were able to lift during the final three sets was significantly greater after cold water immersion compared with active recovery. The average load lifted during the final three sets was not significantly different after cold water immersion compared with the average load recorded during the baseline testing (P > 0.05).

**Muscle temperature.** Muscle temperature increased consistently in all participants after the resistance exercise (P < 0.05). In contrast, the changes in muscle temperature during and after cold water immersion and active recovery were more variable. Individual responses are shown in Fig. 2, A and B. During cold water immersion, muscle temperature decreased by up to 12°C below postexercise values in some participants, whereas in others it only decreased by 1–2°C. During active recovery, muscle temperature increased by 1–2°C above postexercise values in some participants. In other participants, muscle temperature was 3–4°C below postexercise values at the start of active recovery and then only increased by ~1°C. After active recovery, for some inexplicable reason, muscle temperature rose unusually high (i.e., 41°C) in some participants. These participants were therefore excluded from further analysis of muscle temperature. Group data for five participants in the cold water immersion and active recovery trials are shown in Fig. 2C. In these participants, muscle temperature did not change significantly after cold water immersion, whereas it increased significantly during active recovery (P < 0.05) and remained elevated for 2 h. Muscle temperature was significantly higher (P < 0.05) between the 10th and 70th minute of the recovery period after active recovery compared with cold water immersion.

**Blood gases, pH, and lactate.** Blood lactate concentration was higher than preexercise values after the resistance exercise (P < 0.05). It returned to preexercise values within 15 min after active recovery, whereas it remained significantly higher than preexercise values for 2 h after cold water immersion (P < 0.05; Fig. 3A). Blood pH was lower than preexercise values at all time points (Table 2).

### Table 2. Changes in peak force and RFD during isometric squats before, immediately after, and 2 and 4 h postexercise for CWI and ACT conditions

<table>
<thead>
<tr>
<th>Time</th>
<th>Before exercise</th>
<th>Immediately after exercise</th>
<th>2 h postexercise</th>
<th>4 h postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak force, kN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWI</td>
<td>2.3 ± 0.6</td>
<td>2.1 ± 0.4*</td>
<td>2.2 ± 0.7</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>ACT</td>
<td>2.1 ± 0.4*</td>
<td>2.3 ± 0.5</td>
<td>2.8 ± 0.68</td>
<td></td>
</tr>
<tr>
<td><strong>RFD 0–100 ms, N/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWI</td>
<td>972 ± 135</td>
<td>887 ± 151*</td>
<td>954 ± 243*</td>
<td>958 ± 199</td>
</tr>
<tr>
<td>ACT</td>
<td>881 ± 128*</td>
<td>1,026 ± 204*</td>
<td>1,007 ± 202</td>
<td></td>
</tr>
<tr>
<td><strong>RFD 0–200 ms, N/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWI</td>
<td>1,015 ± 128</td>
<td>938 ± 147</td>
<td>992 ± 246</td>
<td>996 ± 212</td>
</tr>
<tr>
<td>ACT</td>
<td>1,043 ± 165</td>
<td>1,062 ± 211</td>
<td>1,037 ± 194</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. RFD, rate of force development. Baseline strength and power data were recorded during the third familiarization session. See Experimental trials in Methods for details. *Significant difference from preexercise (P < 0.05).
values after the resistance exercise ($P < 0.05$; Fig. 3B). It then increased above preexercise values after active recovery but not after cold water immersion. Between 15 min and 2 h after cold water immersion, it was lower compared with active recovery. Venous blood CO$_2$ saturation followed a similar trend to blood pH (Fig. 3D). Venous blood O$_2$ saturation did not change after the resistance exercise. However, it decreased below preexercise values after cold water immersion and remained significantly lower compared with the active recovery trial for the next 2 h (Fig. 3C).

**Muscle damage, swelling, and soreness.** Plasma myoglobin concentration increased above preexercise values from 2 to 4 h after both cold water immersion and active recovery trials ($P < 0.05$; Fig. 4A). It was significantly lower after cold water immersion compared with active recovery at each time point ($P < 0.05$, $d$: −1.0 to −1.4, −51% to −139%).
Lower and upper thigh volume changed over time ($P < 0.001$; Table 3). Both lower and upper thigh volume returned to preexercise values within 1 h after cold water immersion ($P < 0.05$) and remained stable over the remaining 5 h. In contrast, lower thigh volume remained above preexercise values after active recovery ($P < 0.05$). Upper thigh volume displayed a similar trend after active recovery. Lower thigh volume ($P < 0.05$, $d = 0.4$, $2.2\%$, $0.1$ cm) and upper thigh volume ($P < 0.05$, $d = 0.2$, $-2.7$, $-0.1$ cm) were significantly lower from 2 to 6 h after cold water immersion compared with active recovery.

Muscle soreness while standing upright and squatting at 90°C changed significantly over time ($P < 0.001$), increasing preexercise to postexercise, before gradually decreasing toward preexercise values over 6 h. Soreness while squatting was significantly lower after cold water immersion after 5 h ($P = 0.036$, $d = -0.4$, $-2.2\%$, $-0.1$ cm) and 6 h ($P = 0.011$, $d = -0.6$, $-37\%$).

**ET-1 and IL-6.** Serum ET-1 concentration decreased after the active recovery trial ($P < 0.05$) but did not change after the cold water immersion trial. It was below preexercise values at 0.25 h and 0.5 h after active recovery ($P < 0.05$). There were no differences in ET-1 between trials at any time point ($P > 0.05$; Fig. 4B).

Plasma IL-6 concentration was significantly higher than preexercise values from 0.25 to 2 h after both cold water immersion and active recovery trials ($P < 0.05$; Fig. 4C). It was significantly higher at 1.5 h ($P = 0.028$, $d = +1.5$, $+122\%$) and 2 h ($P = 0.038$, $d = +1.4$, $+98\%$) after cold water immersion compared with active recovery.

**DISCUSSION**

In the present study, we investigated how cold water immersion influences the recovery of maximal and submaximal muscle function after high-intensity resistance exercise. Contrary to our hypothesis, compared with active recovery, cold water immersion did not alter recovery of maximal strength or countermovement jump performance. However, it did enhance recovery of submaximal muscle function during a high-intensity resistance exercise test. Cold water immersion also substantially reduced muscle temperature, muscle soreness and swelling, venous $O_2$ saturation, and plasma myoglobin concentration compared with active recovery. Surprisingly, cold water immersion did not alter serum ET-1 concentration, whereas it induced a greater increase in plasma IL-6 concentration compared with active recovery. These findings add to the existing knowledge of the performance benefits and physiological effects of cold water immersion after exercise.

Contrary to our hypothesis, cold water immersion did not enhance recovery of maximal muscle function, as measured by jump performance and isometric strength. Pointon et al. (39) also reported no significant effects of applying ice packs on recovery of maximal isometric torque 2 h after eccentric exercise. In contrast, Vaile et al. (45) observed that cold water immersion enhanced recovery of peak force during isometric squats and peak power during jump squats 24 h after eccentric exercise. Fonda and Sarabon (9) also noted that 3 min of exposure to extremely cold air ($-140$ to $-190^\circ C$) assisted recovery of muscle power during jump squats and counter-
movement jumps 1 h after eccentric exercise. All other studies have failed to demonstrate any benefit of cryotherapy on recovery of maximal strength 24 h or more after eccentric exercise (6, 8, 16, 18, 20, 37, 40) and plyometrics (14, 19, 24). The results of the present study are not directly comparable with these other studies because the exercise was not exclusively eccentric in nature and participants were already familiar with resistance exercise. Consequently, the muscle damage after exercise was probably less severe in this study compared with the studies described above. Nevertheless, our findings suggest that cryotherapy generally does not influence any metabolic or neuromuscular factors that reduce maximal strength and/or power after exercise [e.g., impaired Ca$^{2+}$ release from the sarcoplasmic reticulum (17)].

In support of our hypothesis, cold water immersion enhanced recovery of submaximal muscle function. Participants in the present study were able to lift a greater average and total load during the final three sets of the submaximal muscle function test after cold water immersion compared with active recovery. The obvious implication of this finding is that cold water immersion may assist athletes who sometimes need to train (or compete) twice within the same day. In contrast with our findings, two other studies (13, 23) discovered that cold water immersion did not enhance the total number of squats that participants could perform or the average power during each squat 24 and 48 h after resistance exercise. There are two obvious differences that may account for these conflicting findings. First, in these other studies (13, 23), participants only performed 4 sets of up to 10 squat exercises, whereas participants in our study performed 6 sets of up to 10 squat exercises. Second, in these other studies (13, 23), submaximal muscle function was tested at 24 and 48 h after exercise, whereas we tested submaximal muscle function 6 h after exercise. Therefore, the benefits of cold water immersion may depend on how and/or when submaximal muscle function is tested.

Cold water immersion elicited various physiological responses, some of which could explain the improvement in submaximal muscle function. We measured muscle temperature continuously during cold water immersion and for a further 2 h. Intramuscular temperature increased by ~3–4°C after resistance exercise. After cold water immersion, it was ~4.0°C below preexercise values and ~7°C below postexercise values. Because of substantial interindividual variations, these changes were not statistically significant. Muscle temperature then returned to preexercise values at 20–25 min after cold water immersion. The first 30 min after tissue injury is recognized as a potential window of opportunity to treat muscle injuries (32). A decrease in muscle temperature during this period can reduce secondary tissue damage (33). We did not assess secondary tissue damage directly. However, we found that cold water immersion significantly reduced plasma myoglobin concentration after exercise. This finding provides tentative evidence that cold water immersion may have minimized secondary tissue damage. This result contrasts with most other research indicating no significant effect (9, 13, 14, 16, 18, 19, 23, 24, 39, 40, 45) or an increase (12, 42) in plasma myoglobin concentration or creatine kinase activity in response to cryotherapy after eccentric or resistance exercise. Differences in the extent of muscle damage between resistance exercise and eccentric exercise, the timing of blood collection, or the timing of cryotherapy treatments after exercise could partially account for this disparity. Cold water immersion also reduced muscle soreness and swelling, which may have alleviated feelings of discomfort, thereby allowing participants to perform better during the last three sets of the submaximal exercise test.

Cold water immersion may also benefit recovery from exercise by inducing vasoconstriction and restricting the infiltration of inflammatory cells into muscle (28). We measured the serum concentration of ET-1 to determine whether this potent vasoconstrictor might explain previous observations that cold water immersion reduces blood flow to the limbs (46) and in skeletal muscle (22, 31). Contrary to our hypothesis, serum ET-1 concentration did not increase significantly after cold water immersion. This result was somewhat surprising, con-
local metabolic activity (22). To determine if changes in muscle tissue oxygenation after cold water immersion alter systemic O$_2$ supply/demand, we measured changes in venous blood O$_2$ saturation. We observed that cold water immersion substantially reduced venous blood O$_2$ saturation to between 25% and 30%, whereas it raised venous blood CO$_2$ saturation. These effects persisted throughout the initial 2 h of recovery from exercise. The decrease in venous blood O$_2$ saturation that occurred after cold water immersion was similar in magnitude to the decrease that occurs at the onset of exercise (7). This decline in venous blood O$_2$ saturation therefore likely represents a genuine physiological response to cold water immersion. For example, it might reflect an increase in O$_2$ extraction in skeletal muscle after cold water immersion. Vascular occlusion reduces venous blood O$_2$ saturation (49) and increases motor unit activity and the discharge rate of high-threshold units in skeletal muscle (35). Furthermore, during muscle contractions with vascular occlusion, the integrated electromyogram correlates with muscle tissue oxygenation ($r = 0.562, P < 0.05$) (47). Thus, it is possible that by reducing O$_2$ availability, cold water immersion stimulated the recruitment of type II motor units, which could also possibly account for the improvement in performance during the last three sets of the submaximal exercise test.

**Perspectives and Significance.**

In designing the present study, we aimed to address some of the knowledge gaps and limitations of previous research in the broader field of cryotherapy. We did this through the following means: 1) using traditional resistance exercise as an exercise protocol to simulate common training practices of athletes, 2) measuring both maximal and submaximal muscle function during the early recovery period, 3) using active recovery as a control treatment, and 4) using a randomized cross-over design to minimize any potential series order effects and interindividual variations. We acknowledge some limitations to the present study. First, we only recorded muscle temperature superficially within skeletal muscle (i.e., 1 cm). The reasons for the substantial individual variation in changes in muscle temperature during and after recovery interventions are not immediately obvious. The intensity of active recovery and adipose tissue thickness at the site that we measured muscle temperature varied between individuals. However, muscle temperature...
did not correlate with either exercise intensity or adipose tissue thickness. Second, we did not assess muscle blood flow or O₂ saturation within the muscle. Finally, we did not collect muscle tissue to examine in greater detail the local mechanism(s) by which cold water immersion may have enhanced recovery from exercise. Despite these limitations, our findings are strengthened by the nature of the research design. Our finding that cold water immersion allowed participants to perform more volitional work hints at some central benefits of cold water immersion. Whether cold water immersion provides more than a simple “placebo” effect remains a contentious issue (4, 5). “Central” perceptions of better recovery may play more than a simple “placebo” effect remains a contentious issue. “Central” perceptions of better recovery may play a more dominant role than “peripheral” physiological factors in the capacity for athletes to recover from exercise. If cold water immersion does allow athletes to undertake greater workloads during subsequent training sessions, then this may lead to better training adaptations. Alternatively, cold water immersion could also reduce training adaptations (11, 48) by attenuating some of the key biochemical and molecular processes that underpin local adaptations in skeletal muscle, including protein synthesis, mitochondrial biogenesis, and angiogenesis. Future studies in the field of cold water immersion should focus on addressing the central versus peripheral effects and acute versus chronic effects of cold water immersion.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


Chapter 3

In chapter 2, cold water immersion induced marked physiological effects over the first 2 hours following cold water immersion, for example, upon venous blood gas characteristics. These physiological effects may be attributed to peripheral and/or central hemodynamic responses, which may in turn, effect muscle oxygenation at the microvascular level. This chapter firstly explores the acute peripheral and central hemodynamic responses to cold water immersion i.e. microvascular blood volume vs. cardiac output, and secondly investigates how contraction-induced muscle oxygen consumption/delivery is influenced by hemodynamic manipulation. Lastly, these physiological responses are investigated alongside muscle function to gauge the mechanistic potential of such responses.

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Effects of cold water immersion on central and muscle hemodynamics following resistance exercise

Llion A Roberts¹,², Makii Muthalib³, Jamie Stanley¹,²,⁴, Glen Lichtwark¹, Kazunori Nosaka⁵, Jeff S Coombes¹, Jonathan M Peake²,⁶

¹ The University of Queensland, School of Human Movement Studies, Brisbane, Australia
² Centre of Excellence for Applied Sport Science Research, Queensland Academy of Sport, Brisbane, Australia
³ Movement to Health Laboratory, Euromov, University of Montpellier, Montpellier, France
⁴ Physiology Department, South Australian Sports Institute, Adelaide, Australia
⁵ School of Exercise and Health Sciences, Edith Cowan University, Joondalup, Australia
⁶ School of Biomedical Sciences and Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Corresponding author:
Jonathan Peake
Institute for Health and Biomedical Innovation
Kelvin Grove, QLD 4059
Brisbane, Australia
Email: jonathan.peake@qut.edu.au
Phone: +61 7 3138 6140
ABSTRACT

The effect of lower body cold water immersion on central hemodynamics, tissue temperature, muscle function and tissue oxygenation following resistance exercise was investigated. Using a randomized, cross-over design, 10 physically active men performed 10 sets of 20 maximal unilateral knee extensions, followed by one of two recovery interventions: 10 min of cold water immersion at 10°C (CWI), or 10 min active recovery (low-intensity cycling; ACT), followed by rest at room temperature for 70 min. Cardiac output (using impedance cardiography), heart rate and cardiac parasympathetic activity, intramuscular temperature (18 mm depth) and skin temperature of quadriceps, and muscle oxyhemoglobin (SmO$_2$) (using near infra-red spectroscopy) were measured before exercise, during the recovery interventions, and over the 70 min recovery period. Maximal isometric torque and isokinetic work were also measured before exercise and during the recovery period. Heart rate (37.4 ± 11 bpm) stroke volume (14.5 ± 9.2 ml), cardiac output (4.7 ± 2.4 l), muscle temperature (9.4 ± 5.8 °C) and skin temperature (10.2 ± 5.5 °C) were significantly ($p < 0.05$) lower after CWI versus ACT. Heart rate (10.1 to 19.9 %) and cardiac output (11.2 to 26.8 %) remained significantly lower throughout the recovery period after CWI. Restoration of cardiac parasympathetic activity was faster following CWI versus ACT (by 5 versus 20 min). SmO$_2$ desaturation time during maximal voluntary contractions (MVCs) was significantly faster (12.7 to 16.5 %) whereas SmO$_2$ resaturation time after MVCs was significantly slower (30.7 to 48.7 %), following CWI versus ACT. MVC peak torque was significantly greater 20 min and 40 min after CWI vs ACT ($p < 0.05$; 17.8 to 21.2 %), whereas there was no significant difference in isokinetic work. In conclusion, CWI alters central and muscle hemodynamics, which may have important implications for blood flow and metabolism in skeletal muscle during recovery from resistance exercise.

Key words: Cryotherapy, muscle oxygenation, blood flow, recovery.
INTRODUCTION

Cryotherapy treatments such as cold water immersion, ice baths and ice massage are popular strategies to recover from exercise. This popularity is partly related to the psychological benefits of these treatments, such as lower ratings of fatigue and muscle soreness (Versey et al., 2013). Some studies have also reported benefits of cold water immersion treatments on muscle function and various aspects of exercise performance (for review see (Versey et al., 2013). Despite its popularity and anecdotal support, the physiological mechanisms underlying the effects of cold water immersion during the post-exercise period are not yet well established.

Cold water immersion is purported to promote recovery by exerting hydrostatic pressure on the body and altering tissue temperature (Wilcock et al., 2006). Hydrostatic pressure is proposed to assist post-exercise recovery by stimulating hemodynamic changes that aid the removal of metabolites in skeletal muscle (Bleakley & Davison, 2010). Under resting conditions, regardless of temperature, water immersion increases cardiac output and stroke volume (Farhi & Linnarsson, 1977; Weston et al., 1987; Bonde-Petersen et al., 1992; Gabrielsen et al., 2000; Shiraishi et al., 2002), venous pressure (Gabrielsen et al., 2000) and systolic blood pressure (Bonde-Petersen et al., 1992; Gabrielsen et al., 2000), whereas it generally reduces heart rate (Farhi & Linnarsson, 1977; Weston et al., 1987; Gabrielsen et al., 2000; Shiraishi et al., 2002). Changes in total peripheral resistance (Bonde-Petersen et al., 1992; Shiraishi et al., 2002; Muller et al., 2012), venous (Barcroft & Edholm, 1943) and arterial blood flow (Blyden et al., 1989; Gregson et al., 2011) following water immersion at rest are more variable. Some of this variation may be due to differences in water temperature and methods used to assess vascular conductance. These hemodynamic responses may also occur independently of hydrostatic pressure in response to surface cooling of the skin (Knight & Londeree, 1980; Wilson et al., 2007; Selkow et al., 2012). A decrease in tissue temperature is proposed to enhance post-exercise recovery by reducing cellular metabolism and secondary ischemic or enzymatic damage in skeletal muscle (Bleakley et al., 2012). Immersion in cold water (Gregson et al., 2011; Rupp et al., 2012) is effective for reducing muscle temperature under resting conditions. Muscle temperature decreases for a longer period of time after cold water immersion when compared with only cooling the skin and underlying tissue using ice (Rupp et al., 2012).

In contrast with this extensive research under resting conditions, less is known about how cold water immersion influences hemodynamics and thermoregulation after exercise. The findings from research on hemodynamic and thermoregulatory responses to
thermoneutral water immersion at rest are not necessarily applicable to the post-exercise context. Exercise and/or passive heating induce fundamental physiological changes that alter central hemodynamic and thermoregulatory responses to subsequent cold exposure (Ebbesen et al., 1992; Probst et al., 1997; Castellani et al., 1999; Cui et al., 2010). Furthermore, in some of the studies of thermoneutral water immersion at rest, the period of water immersion was longer (Blyden et al., 1989; Muller et al., 2012), and/or the water temperature was higher (i.e., ≥30°C) (Farhi & Linnarsson, 1977; Weston et al., 1987; Blyden et al., 1989; Gabrielsen et al., 2000) than is typically used to recover from exercise (Bleakley & Davison, 2010).

Cold water immersion after exercise accelerates heart rate recovery by stimulating parasympathetic innervation of the heart (Stanley et al., 2012). However, fewer studies have investigated how cold water immersion influences other central hemodynamic variables such as cardiac output and mean arterial blood pressure after exercise (Mawhinney et al., 2013; Stanley et al., 2014). Cold water immersion after exercise reduces peripheral hemodynamics such as muscle blood flow (Mawhinney et al., 2013), limb blood flow (Vaile et al., 2010) and muscle blood volume (Ihsan et al., 2013). Other studies have also reported that cold water immersion reduces skin, core and muscle temperature after exercise (Peiffer et al., 2009; Gregson et al., 2013; Mawhinney et al., 2013; Broatch et al., 2014; Ihsan et al., 2014; Stanley et al., 2014). Previous research on the hemodynamic and thermoregulatory effects of cold water immersion during recovery from exercise has been restricted to intermittent or continuous cycling or running (Peiffer et al., 2009; Vaile et al., 2010; Gregson et al., 2013; Mawhinney et al., 2013; Broatch et al., 2014; Ihsan et al., 2014). Autonomic regulation of heart rate is different during recovery from endurance exercise and resistance exercise (Heffernan et al., 2006). Accordingly, the effects of cold water immersion on hemodynamics may also differ during recovery from resistance exercise compared with endurance exercise. Previous research has used near infra-red spectroscopy (NIRS) to measure muscle oxyhemoglobin saturation (SmO₂) as a measure of tissue oxygenation, and total hemoglobin (tHb) concentration as a measure of muscle blood volume after cold water immersion (Ihsan et al., 2013; Tseng et al., 2013; Stanley et al., 2014). Changes in SmO₂ provide information on the balance between O₂ consumption and delivery in muscle (Ryan et al., 2013). To distinguish O₂ consumption from O₂ delivery, it is necessary to block O₂ delivery to muscle through arterial occlusions (Ryan et al., 2013) maximal voluntary isometric contractions (Muthalib et al., 2010). This approach provides a means to obtain novel information on how cold water immersion
might affect muscle O$_2$ consumption and reactive hyperemia during and following muscle contraction (Ferrari et al., 2011).

The aim of this study was to compare the effects of cold water immersion versus active recovery on central (cardiac output, heart rate, cardiac parasympathetic activity) and muscle (muscle oxygenation and blood volume) hemodynamics, thermoregulation (intramuscular and skin temperature), and muscle function following intense resistance exercise. It was hypothesized that compared with active recovery, cold water immersion would reduce central and muscle hemodynamics and tissue temperature, whereas it would enhance recovery of muscle function after resistance exercise.

**METHODS**

**Subjects**

Ten recreationally active men (mean ± SD age: 21.4 ± 2 years, height 1.8 ± 0.1 m, body mass 83.7 ± 14.8 kg) who were familiar with knee extension exercise, and had been resistance training 2 to 3 times a week for the previous 12 months volunteered to participate. Experimental procedures and risks were explained to the participants before they provided their informed consent to take part in the study. The study was approved by the Human Research Ethics Committee of The University of Queensland.

**Experimental design**

The participants completed a familiarization trial and baseline testing 7 d before the experimental trials. Experimental trials involved unilateral knee extensor exercise with the dominant leg, followed by a 10 min period of cold water immersion or active recovery, and a 70 min recovery period. A 5 min period separated the end of the exercise bout and the beginning of the recovery intervention. Another 5 min period separated the end of the recovery interventions and the beginning of the recovery period. During these periods, the participants moved between the experimental laboratory and a room containing the apparatus for the recovery interventions. Experimental trials were performed 7 d apart, in a randomized and counter-balanced manner. Familiarization, baseline testing and the experimental trials were all performed in a temperature-controlled laboratory (mean ± SD temperature 24.4 ± 0.2°C, humidity 43.5 ± 1.6 %).

**Familiarization trials and baseline testing**

The familiarization session allowed the participants to practice the unilateral leg exercise that they would perform in the experimental trials, and to familiarize themselves
with the requirements of the physiological measurements. Baseline testing involved recording isokinetic torque during 50 maximal knee extensions (details below). The data recorded during this testing were used for comparison with post-exercise values after each of the experimental trials. We did not measure isokinetic torque on the day of each experimental trial because we expected that the 50 maximal knee extensions would cause fatigue prior to the unilateral resistance exercise.

**Experimental trials**

All trials started at 9 a.m. The participants were asked to eat similar food, and drink 10 ml·kg\(^{-1}\) of water in the 2 h prior to each trial. They were asked to avoid consuming stimulants, alcohol, tobacco, antioxidants and nutritional supplementation for 24 h preceding all trials. They were allowed to exercise as normal between trials, but were asked not to do any lower body strength exercise for 48 h prior to each trial. Trials commenced with a 15 min rest period while the apparatus for recording muscle and skin temperature, cardiac output and muscle tissue oxygenation was inserted and/or attached. After the apparatus was set up, pre-exercise data for muscle and skin temperature, cardiac output, heart rate, muscle oxygenation/hemodynamics and isometric strength were collected. The participants then rested for another 5 min before they started the unilateral knee extension exercise (see exercise and performance testing). In the first 10 min after exercise, heart rate variability was measured again. The participants then started one of the recovery interventions (cold water immersion or active recovery). After this intervention, the participants rested for ~70 min while muscle and skin temperature, cardiac output, heart rate, muscle oxygenation/hemodynamics and isometric strength were measured. Resting heart rate variability and resting muscle oxygenation were measured immediately prior to testing isometric strength at 5, 20 and 40 min after the recovery interventions. Isokinetic torque was measured 60 min after the recovery interventions.

To minimise the influence of movement and shivering on cardiac dynamics and NIRS, all trials were firstly performed at ambient room temperature of ~24°C. Participants also kept their torso and exercising leg as still as possible whilst measurements were undertaken (accounting for NIRS measurements taken during, and in response to exercise). Finally, both legs were towel-dried following cold water immersion, and a towel was also applied to the torso for 5 minutes following both recovery therapies.
**Unilateral resistance exercise, isometric strength and isokinetic performance**

All exercise was performed unilaterally on an isokinetic dynamometer (Cybex 6000, CSMI, Stoughton, MA, USA). Unilateral exercise on a dynamometer because the dynamometer provided more data on torque output, and because bilateral exercise is difficult on a dynamometer. The dynamometer position was modified to align the lateral condyle of the femur with the point of rotation, and the seat angle was fixed at 90°. The unilateral exercise bout consisted of 10 sets of 20 maximal isokinetic knee extensions performed using the dominant leg, over a 90° range at a velocity of 90°⋅s⁻¹. The participants rested in the seat of the dynamometer for 2 min between sets. Repetition tempo was set at 0.5 Hz by an audio signal, and knee flexion velocity was set at 250°⋅s⁻¹ to allow passive flexion following each knee extension.

Isometric knee extension strength was measured at a knee joint angle of 70° (full knee extension = 0°). For each contraction, the participants were instructed to extend their knee as forcefully as possible at 70°, and continue pushing against the stop point for the required duration. The participants performed two warm-up contractions, each lasting 5 s, and separated by 90 s. They then rested for another 90 s before they performed a maximal voluntary isometric contraction for 10 s. Maximum isometric torque was recorded from this 10 s contraction. This procedure was repeated before, 5 min, 20 and 40 min after the unilateral exercise bout. The isokinetic performance task was performed during baseline testing (see previous details) and 60 min after the unilateral exercise bout. It consisted of 50 sequential maximal isokinetic knee extensions, using the same range, velocity and tempo as the exercise bout.

All data from the dynamometer were collected at 1,000 Hz using a custom-designed Labview script (Labview, National Instruments Corp., Texas, USA), and stored on a personal computer for offline analysis. The coefficient of variation for peak torque from the 10 s isometric contraction was 2.5%. The coefficient of variation for total work performed over the isokinetic task was 3.2%.

**Recovery interventions**

Recovery interventions consisted of cold water immersion or active recovery. For the cold water immersion therapy, the participants continuously immersed their body (up to the umbilicus) for 10 min in an inflatable bath (iBody, iCool Australia Pty Ltd., Miami, Australia) containing water at 10 ± 0.2°C. Currently, there is little agreement regarding the optimal temperature and duration of cold water immersion to promote recovery from exercise. However, the most common water temperature used in previous cold water...
immersion studies is ∼10°C (Bleakley & Davison, 2010). Furthermore, 10 min of cold water immersion at 8°C significantly reduces intramuscular and rectal temperature under resting conditions (i.e., no prior exercise), while 15 min of immersion at 10°C significantly reduces muscle blood volume and metabolic activity after exercise (Ihsan et al., 2013). In the present study, the participants only immersed their body to the level of their umbilicus, because immersion up to the neck may have interfered with electrodes placed on the torso to estimate stroke volume. Water temperature in the bath was continuously maintained using a circulatory cooling unit (iCool Lite, iCool Australia Pty Ltd., Miami, Australia). For the active recovery therapy, the participants were instructed to exercise on a cycle ergometer (Wattbike®, Wattbike Ltd., Nottingham, UK) for 10 min at a low, self-selected intensity. Active recovery was chosen over a passive control condition, because it is unlikely that athletes would not undertake some activity in recovery from prior exercise. For example, active recovery has long been known to reduce blood lactate concentrations following high intensity exercise (Bond et al., 1991; Ahmaidi et al., 1996). The participants cycled a distance of 3.4 ± 0.3 km, at an average power output of 41.1 ± 10.3 W during active recovery.

**Cardiac dynamics**

Heart rate, stroke volume and cardiac output were measured continuously by impedance cardiography (Physioflow®, Manatec Biomedical, Paris, France). This method detects changes in impedance of alternating low magnitude electrical current between electrodes on the neck (above the supra-clavicular fossa) and the xiphoid process. Calibration was completed at rest by collecting data over 30 cardiac cycles, and inputting data for diastolic and systolic blood pressure measured from the brachial artery using an automated sphygmomanometer (Digital blood pressure monitor, UA-767, A&D Instruments Ltd., UK) over the same period. Blood pressure data were updated 3 min after the completion of the recovery therapy. Data were sampled at 2 beat intervals, and saved on a personal computer for offline analysis. The coefficient of variation when using the Physioflow® at rest was 1.7% for heart rate, 4.1% for stroke volume and 2.9% for cardiac output. The coefficient of variation for post-exercise heart rate, stroke volume and cardiac output following unilateral knee extensor exercise was 5.4% for heart rate, 7.0% for stroke volume and 4.0% for cardiac output.

Heart rate variability was assessed from the time domain by recording the natural logarithm of the square root mean of the sum of the squared differences between adjacent normal R–R intervals (Ln rMSSD) sequential R–R intervals while the participants were
seated. A heart rate monitor (Suunto T6c, Suunto Oy, Vantaa, Finland) was used at a sampling frequency of 1,000 Hz. Ln rMSSD was calculated for heart rate recorded before exercise (PRE), immediately after the resistance exercise (POST), during the recovery interventions (REC), between 0–5 min (5 min), 15–20 min (20 min) and 35–40 min (40 min) during the recovery period, and immediately after the isokinetic task (POST-isokinetic). Respiration rate was not controlled during these measurement periods, because heart rate variability indices of parasympathetic activity are similar during controlled or spontaneous breathing (Bloomfield et al., 2001) and do not influence Ln MSSD (Pentila et al., 2001). Data files were transferred to a personal computer using Suunto Team Manager Software (Suunto T6c, Suunto Oy, Vantaa, Finland). Offline analysis was conducted from 2–5 min of each 5 min interval, and from 2–9 min during the recovery interventions.

All cardiac dynamics were measured in an upright position. The participants were seated on the dynamometer with a fixed hip angle of 90°, and were instructed to sit upright at the same hip angle of ~90° while they completed each recovery therapy. They also stood upright stance while moving between isokinetic dynamometer and the recovery apparatus.

Muscle oxygenation and hemodynamics

Muscle oxygenation (SmO₂) and hemodynamics (tHb) were measured by a portable NIRS system (Portamon®, Artinis Medical Systems, Netherlands). The NIRS device emits light at wavelengths of 760 and 850 nm from three optodes, with an average optode-detector distance of 35 mm. Penetration depth of the light below the skin surface is approximately 17.5 mm, or half the optode-detector distance (Muthalib et al., 2010). These measurements were performed during (10 s) and after (2 min) the maximum isometric voluntary contractions (MVCs), which the participants performed before, 5 min, 20 min and 40 min after the unilateral exercise bouts. These MVCs were used in place of arterial occlusions to differentiate between O₂ consumption and O₂ delivery (Muthalib et al., 2010).

The NIRS probe was placed on the mid-line of the vastus lateralis muscle, at one-third of the linear distance between the superior border of the patella and the inguinal fold. Probe location measurements were recorded and outlined with a marker for re-positioning during the second experimental trial. The NIRS probe was placed in a sealed polythene bag and covered with a black plastic cloth for waterproofing and protection from ambient light. Adipose tissue thickness of the participants was measured by skinfold calipers (Harpenden skinfold caliper, Baty International, West Sussex, UK). Mean ± SD adipose tissue thickness

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was 6.5 ± 3.4 mm, calculated as skinfold thickness divided by two. Therefore, the intramuscular NIRS penetration depth was 11.5 ± 3.4 mm, calculated as 17.5 mm minus adipose tissue thickness. At this penetration depth, the corresponding NIRS signal mainly reflects the metabolic and hemodynamic changes of the muscle (Ferrari et al., 2004). The NIRS method is both reliable and valid for the measurement of muscle oxidative metabolism (Ryan et al., 2013). The coefficient of variation for test-retest reliability of resting SmO$_2$ was 2.3% in this investigation.

Data were recorded on-line at 10 Hz using native software (Oxysoft V.2.1.6; Artinis Medical Systems, Netherlands). The software calculates changes in light absorption at the different wavelengths, and converts them to relative concentrations of oxyhemoglobin (O$_2$Hb) and deoxyhemoglobin (HHb) using the modified Lambert Law to correct for light scattering within the tissue. Total hemoglobin (tHb) was calculated as O$_2$Hb + HHb. The balance between oxygen delivery and consumption was represented by the tissue oxygenation index (SmO$_2$), calculated as $[(O_2Hb / (O_2Hb + HHb)] \times 100$ by spatial resolved spectroscopy. Off-line analysis was performed using the same software.

tHb and SmO$_2$ were continuously recorded during all trials. Pre-contraction SmO$_2$ and tHb were calculated as the mean from $-4$ to $-1$ s prior to the onset of the contraction. For the purpose of extracting data, the start of the contraction was identified as the time when tHb was lower than pre-contraction tHb + (2 × SD) for a period of at least 1 s. The end of the contraction was identified as the time when tHb was higher than mean tHb during the contraction + 2 × SD. SmO$_2$ maximum was identified as the time corresponding to the maximum SmO$_2$ % within 90 s following the end of the contraction. The following variables were calculated (see Figure 3.1):

(i) Contraction duration, calculated from tHb changes.

(ii) SmO$_2$ ½ desaturation time (½ DT), which corresponds to the period of time between the start of the contraction until SmO$_2$ reaches 50% of the difference between baseline SmO$_2$ (i.e., pre-contraction) and the minimum SmO$_2$ during the contraction. A longer duration ½ desaturation time for a similar minimum SmO$_2$ represents a slower oxyhemoglobin desaturation rate, indicating that O$_2$ consumption is better matched by O$_2$ delivery (Ferrari et al., 2011).

(iii) Minimum $\Delta$SmO$_2$ desaturation amplitude, which corresponds to the difference minimum SmO$_2$ during the contraction. Lower SmO$_2$ minimum values indicate greater O$_2$ consumption relative to O$_2$ delivery (Ferrari et al., 2011).
(iv) SmO₂ half-recovery time (½ RT), which corresponds to the time required (from the end of the contraction) for SmO₂ to reach 50% of SmO₂ maximum. A lower value indicates greater O₂ delivery relative to O₂ consumption.

(v) Time from SmO₂ half-recovery time to SmO₂ maximum. A lower value indicates greater O₂ delivery relative to O₂ consumption.

(vi) Time to full SmO₂ recovery, which corresponds to the time difference between SmO₂ half-recovery time and when SmO₂ returned to baseline after the contraction. A lower value indicates greater O₂ delivery relative to O₂ consumption.

**Temperature measurement**

Intramuscular temperature was measured using a fine-wire implantable probe (T204E, Physitemp Instruments Inc, NJ, USA), while skin temperature was measured with a surface probe (SS-1, Physitemp Instruments Inc, NJ, USA). Temperature was logged at a frequency of 1 Hz using a portable data logger (SQ2020, Grant instruments, UK), and transferred to a portable computer for analysis. An 18 gauge needle was used to insert the implantable probe to a depth of 18 mm beneath the skin surface, 5 cm superior to the NIRS probe. This depth was chosen to measure superficial muscle temperature, and to correlate with the 17.5 mm sub-cutaneous working depth of the NIRS probe. Once the intramuscular temperature probe was at the required depth, the needle was removed, leaving the probe in place. The skin temperature probe was placed on the quadriceps immediately next to the medial border of the NIRS device. Data were averaged for 1 min

![Figure 3.1](image.png)

**Figure 3.1.** A schematic illustration of NIRS variables during, and following an isometric maximal voluntary contraction. Grey section represents the 10 sec contraction period.
intervals before and after the exercise bout, during the recovery interventions, and every 2 min for the recovery period.

**Statistical analysis**

Statistical analysis was conducted using the Statistical Package for Social Sciences program (V.21, IBM, New York, USA). Heart rate variability was initially log transformed. All data were initially assessed for normality using the Shapiro-Wilks formula. All measures were assessed by two-factor repeated measures ANOVA. When significant time or condition \( \times \) time interaction effects were evident, paired t-tests were used to compare changes over time and between trials, and the false discovery rate was used to correct p values for these multiple comparisons. To complement these statistical comparisons, Cohen’s effect size (d) was calculated to illustrate the magnitudes of changes or differences in muscle function and physiological variables over time and between the trials. Effect sizes were assessed as 0.2 = small effect, 0.5 = moderate effect, and \( \geq 0.8 \) = large effect. All data are presented as mean ± standard deviation (SD). Significance was set at a level of \( p < 0.05 \).

**RESULTS**

**Exercise bout**

The total work completed during the unilateral exercise bout was not different (\( p = 0.2 \)) between the active recovery (22.2 ± 4.4 kJ) and cold water immersion conditions (22.8 ± 5.9 kJ).

**Central hemodynamics**

Changes in heart rate were different between the cold water immersion and active recovery conditions (condition \( \times \) time interaction effect \( p < 0.001 \), \( F = 39.87 \)). During cold water immersion, heart rate remained higher than pre-exercise values (\( p < 0.05 \)) over the first 2 min, but then returned to pre-exercise values after 5 min (Figure 3.2A). It subsequently dropped below pre-exercise values again between 50 and 70 min after cold water immersion (\( p < 0.05 \)). Conversely, it remained above pre-exercise values during the full recovery period following active recovery (\( p < 0.05 \)). Heart rate was lower following cold water immersion compared with active recovery for the entire recovery period (\( p < 0.05 \); Cohen’s effect size \( d = -0.7 \) to \(-3.6 \)) and following the isokinetic task (\( p < 0.001 \); \( d = -1.9 \)).
Changes in stroke volume were different between the cold water immersion and active recovery conditions (interaction effect $p < 0.001$, $F = 3.87$). Stroke volume gradually decreased during cold water immersion, and tended to be lower than pre-exercise values from 7−10 min ($p = 0.1$ to 0.06; $d = -0.3$ to $-0.5$) (Figure 3.2B). By contrast, it tended to increase above pre-exercise values during active recovery ($p = 0.08$ to 0.03; $d = 0.4$ to 0.6). Stroke volume was lower from 2−10 min during cold water immersion compared with active recovery ($p \leq 0.038$; $d = -0.4$ to $-1.0$). Following both cold water immersion and active recovery, it was not significantly different than pre-exercise values during the remainder of the recovery period ($p > 0.05$). It was also not significantly different between the two conditions during this period.

Changes in cardiac output were different between the cold water immersion and active recovery conditions (interaction effect $p < 0.001$, $F = 23.41$). Consistent with the decrease in stroke volume, cardiac output decreased during cold water immersion, and was lower than pre-exercise values from 8 to 10 min ($p < 0.05$) (Figure 3.2C). Similar to heart rate, cardiac output dropped below pre-exercise values again between 30 to 60 min after cold water immersion ($p < 0.05$). Conversely, it increased moderately during active recovery, and remained above pre-exercise values until 20 min after active recovery ($p < 0.05$). It was lower during and after cold water immersion compared with active recovery ($p < 0.01$; $d = -0.8$ to $-2.9$).
Figure 3.2. Mean (± SD) heart rate, stroke volume and cardiac output before (PRE) and after (POST) resistance exercise, during the recovery interventions (grey section), during the recovery period, and following the dynamic task (PD). Vertical dashed line represents the beginning of the recovery period (0 min) in the cold water immersion (CWI) and active recovery (ACT) conditions. *p < 0.05 vs pre-exercise. #p < 0.05 between conditions.

Muscle oxygenation and hemodynamics during and after recovery interventions

Changes in muscle oxygenation (SmO$_2$) and hemodynamics (tHb) during the recovery interventions were different between the two conditions (interaction effect $p < 0.01$, SmO$_2$ $F = 2.703$, tHb $F = 4.25$). During cold water immersion, SmO$_2$ did not change ($p > 0.05$), whereas it progressively decreased during active recovery ($p = 0.065$ to 0.004)
(Figure 3.3A). $\text{SmO}_2$ was lower from 3–10 min during active recovery compared with cold water immersion ($p \leq 0.038$; $d = -0.4$ to $-1.2$). During cold water immersion, $tHb$ increased during the first 4 min (Figure 3.3B). It then decreased slightly over the remaining 6 min, but still remained high compared with the first minute of cold water immersion ($p \leq 0.038$). $tHb$ progressively increased during active recovery ($p \leq 0.017$). It was higher from 2–6 min during cold water immersion compared with active recovery ($p = 0.037$ to 0.046; $d = 0.9$ to 1.1), and tended to remain higher at minutes 7 ($p = 0.067$; $d = 0.7$) and 8 ($p = 0.098$; $d = 0.5$). After the recovery interventions, $\text{SmO}_2$ did not change significantly compared with pre-exercise values (time effect $p = 0.093$) (Figure 3.3C). Changes in $tHb$ after the recovery interventions were different between the two conditions (interaction effect $p < 0.001$) (Figure 3.3D). $tHb$ was lower than pre-exercise values 5 min ($p = 0.003$) and 20 min ($p = 0.033$) after cold water immersion, whereas it was higher than pre-exercise values 40 min ($p = 0.037$) after active recovery. $tHb$ was lower at 20 min ($p = 0.035$; $d = 1.2$) and 40 min ($p = 0.031$; $d = 0.8$) after cold water immersion compared with active recovery.

**Figure 3.3.** Mean (± SD) quadriceps muscle oxygenation ($\text{SmO}_2$) and blood volume ($tHb$) changes during (A and B) and following (C and D) the cold water immersion (CWI) and active (ACT) recovery interventions. Data are. *$p < 0.04$ vs minute 1 (A and B) or PRE (C and D). #$p < 0.05$ between conditions.
Muscle oxygenation and hemodynamics during and after MVCs

Changes in SmO2 kinetics during and after the isometric contractions were also different between the two conditions (interaction effects $p = 0.003$ to 0.047, $F = 4.58$ TO 5.91). SmO2 ½ desaturation time (SmO2 ½ DT) was calculated as the period of time between the start of the maximal isometric contraction until SmO2 reached 50% of the difference between baseline SmO2 (i.e., pre-contraction) and the minimum SmO2 during the contraction (see Figure 3.1). Compared with pre-exercise values, SmO2 ½ DT did not change significantly after exercise in the cold water immersion condition ($p = 0.155$ to 0.631). By contrast, it was longer compared with pre-exercise values at 20 min ($p = 0.001$) and 40 min ($p = 0.003$) after exercise in the active recovery condition (Figure 3.4A). ½ DT was also longer at 20 min ($8.3 \pm 1.4$ versus $7.3 \pm 1.8$ s; $p = 0.028$; $d = 0.9$).

Figure 3.4. Mean (± SD) changes in quadriceps muscle oxygenation (SmO2) kinetics (a) and amplitude (b) during isometric contractions. SmO2 ½ DT, half desaturation time. SmO2 ½ RT, half recovery time. Full rec, full recovery to pre-contraction TOI values. Data to the left of the dotted line are plotted on the left y-axis; data to the right of the dotted line are plotted on the right y-axis. CWI, cold water immersion; ACT, active recovery. *$p < 0.05$ vs pre-exercise. #$p < 0.05$ between conditions.
SmO$_2$ half-recovery time ($\frac{1}{2}$ RT) was calculated as the time required (from the end of the contraction) for SmO$_2$ to reach 50% of its maximum after the contraction (see Figure 3.1). Compared with pre-exercise values, SmO$_2$ $\frac{1}{2}$ RT tended to be longer at 20 min ($p = 0.1$) and 40 min ($p = 0.052$) after cold water immersion (Figure 3.4A). By contrast, it was not significantly different than pre-exercise values at 20 min ($p = 0.21$) and 40 min ($p = 0.36$) after active recovery. SmO$_2$ $\frac{1}{2}$ RT was longer at 5 min (13.3 ± 6.7 vs 8.8 ± 3.0 s; $p = 0.047$; $d = 0.5$), 20 min (14.3 ± 7.1 vs 9.2 ± 3.1 s; $p = 0.033$; $d = 0.5$) and 40 min (15.3 ± 8.3 vs 9.5 ± 3.0 s; $p = 0.046$; $d = 0.6$) after cold water immersion compared with active recovery.

Compared with pre-exercise values, the period between SmO$_2$ half-recovery time and SmO$_2$ maximum ($\frac{1}{2}$ RT-max) did not change significantly after cold water immersion ($p \geq 0.4$) (Figure 3.5A). By contrast, it was shorter than pre-exercise values at 5 min ($p = 0.014$), and tended to remain shorter at 20 min ($p = 0.071$) after active recovery. $\frac{1}{2}$ RT-max was longer at 5 min ($p = 0.005$; $d = 1.2$), 20 min ($p = 0.014$; $d = 1.8$), and 40 min ($p = 0.046$; $d = 0.5$) after cold water immersion compared with active recovery. The period between SmO$_2$ half-recovery time and when SmO$_2$ returned to baseline (full recovery) followed a similar trend to the changes in $\frac{1}{2}$ RT-max (Figure 3.4A).

SmO$_2$ amplitude was calculated as the difference between SmO$_2$ maximum after the maximal isometric contraction, and SmO$_2$ minimum during the maximal isometric contraction. SmO$_2$ amplitude decreased significantly after exercise (time effect $p < 0.001$). It was lower than pre-exercise values ($p < 0.05$) at 5 min and 20 min after exercise in both the cold water immersion and active recovery conditions (Figure 3.4B). It also remained lower than pre-exercise values at 40 min after exercise in the cold water immersion trial. SmO$_2$ amplitude was not significantly different between the two conditions.

**Skin and muscle temperature**

Changes in muscle and skin temperatures were different between the cold water immersion and active recovery conditions (both interaction effects $p < 0.001$, $F = 10.61$ for muscle temperature, and $F = 11.9$ for skin temperature). Both muscle and skin temperature were higher than pre-exercise values immediately after the resistance exercise in both conditions ($p < 0.001$) (Figure 3.5). Muscle and skin temperature decreased during cold water immersion, and remained below pre-exercise values for 50 min after cold water immersion ($p < 0.05$). By contrast, muscle and skin temperature remained above pre-exercise values for 60 min after active recovery ($p < 0.05$). Muscle temperature ($p < 0.05$; $d = −0.9$ to −2.8) and skin temperature ($p < 0.05$; $d = −0.8$ to −3.7)
were lower for the entire recovery period after cold water immersion compared with active recovery. 10.614 11.895

**Figure 3.5.** Mean (± SD) muscle and skin temperatures before (PRE) and after (POST) the exercise bout, during the recovery interventions (grey section), and the recovery period in the cold water immersion (CWI) and active recovery (ACT) conditions. Vertical dashed line represents the beginning of the recovery period (0 min). *p < 0.05 vs pre-exercise. #p < 0.05 between conditions.

**Heart rate variability**

Changes in Ln rMSSD were different between the cold water immersion and active recovery conditions (interaction effect p < 0.001, F = 13.63). Ln rMSSD was lower after the exercise bout in both conditions (p < 0.01; d = −2.4) (Figure 3.6). It rapidly returned towards pre-exercise values during cold water immersion, whereas it remained lower than pre-exercise at 5 min after active recovery (p = 0.003; d = −1.8). It was higher during (p = 0.005; d = 2.4) and after (p = 0.012; d = 1.6) cold water immersion compared with active recovery.
Figure 3.6. Mean (± SD) natural logarithm of the root mean square of successive differences in $R-R$ interval (Ln rMSSD) before (PRE) and after (POST) the exercise bout, during the recovery interventions (REC) and at 5, 20 and 40 min of the recovery period in the cold water immersion (CWI) and active recovery (ACT) conditions. *$p < 0.05$ vs pre-exercise. #$p < 0.05$ between conditions.

**Muscle function**

Changes in peak isometric torque were different between the conditions (interaction effect $p = 0.034$, $F = 3.34$). Peak isometric torque did not change significantly after exercise in the cold water immersion condition (Figure 3.7A). By contrast, it was lower than pre-exercise values at 5, 20 and 40 min ($p < 0.05$) after exercise in the active recovery condition. It was also lower at 20 min ($p = 0.044$; $d = −0.8$) and 40 min ($p = 0.033$; $d = −0.7$) after exercise in the active recovery condition compared with the cold water immersion condition. Mean isometric torque followed a similar trend (Figure 3.7B). The total amount of work completed during the 50 isokinetic contractions was similar at baseline and 60 min post-exercise in both the cold water immersion and active recovery conditions (time effect $p = 0.7$) (Table 3.1). During the 50 contractions, isokinetic torque decreased progressively over time (time effect $p < 0.001$) in both the cold water immersion and active recovery conditions.

**DISCUSSION**

This study provides important new insights into the effects of cold water immersion on central and muscle hemodynamics during the recovery period following resistance exercise. During cold water immersion, heart rate, stroke volume and cardiac output all decreased more rapidly compared with active recovery. In the hour after cold water immersion, heart rate and cardiac output remained lower compared with active recovery.
In the period after cold water immersion, SmO₂ desaturation during MCVs was faster, whereas SmO₂ resaturation after MVCs was slower with active recovery. Despite these central and muscle hemodynamic responses and lower muscle temperature, cold water immersion helped to maintain maximal isometric muscle function after exercise. Collectively, these findings complement and extend existing knowledge, and raise some interesting questions about the physiological effects of cold water immersion after resistance exercise.

This is the first study to investigate the effects of cold water immersion on stroke volume and cardiac output during recovery from resistance exercise. We assessed these hemodynamic variables because cold water immersion is proposed to promote recovery from exercise by increasing central venous pressure through hydrostatic pressure on the body (Wilcock et al., 2006). In the present study, after resistance exercise, cold water immersion...
immersion reduced cardiac output and stroke volume more rapidly compared with active recovery. At the end of cold water immersion, stroke volume and cardiac output were both lower than pre-exercise values. In contrast with our findings, other research generally indicates that cardiac output and stroke volume increase after water immersion at rest without prior exercise (Farhi & Linnarsson, 1977; Weston et al., 1987; Bonde-Petersen et al., 1992; Shiraishi et al., 2002). Several explanations may account for this difference.

In the present study, cardiac output and stroke volume were already relatively high before cold water immersion as a result of prior resistance exercise. Consequently, the hydrostatic effect of water immersion was possibly not sufficient to increase cardiac output and stroke volume further above post-exercise levels (akin to a ‘ceiling effect’). In studies reporting an increase in stroke volume and cardiac output after water immersion, stroke volume was around 60 ml, while cardiac output was 4–5 l/min before immersion (Shiraishi et al., 2002) or under resting control conditions out of water (Farhi & Linnarsson, 1977). By comparison, in the present study, stroke volume was around 85 ± 22 ml, while cardiac output was 8.0 ± 2.2 l/min immediately before of cold water immersion.

Irrespective of whether such a ceiling effect did occur, prior resistance exercise could also have inhibited any increase in stroke volume and cardiac output during cold water immersion. Prior moderate-intensity exercise and/or passive heating attenuate the normal increase in mean arterial pressure, cardiac output, heart rate and diastolic pressure following the cold pressor test (Ebbesen et al., 1992; Probst et al., 1997; Cui et al., 2010). Exercise and/or body heating may inhibit these hemodynamic responses to cold water immersion by reducing the responsiveness of central and peripheral blood vessels to α-adrenergic stimulation (Cui et al., 2002; Wilson et al., 2002). In the present study, prior resistance exercise (and the associated increase in body temperature) may have partially prevented peripheral vasoconstriction during cold water immersion, resulting in less blood returning to the heart, lower stroke volume and lower cardiac output. Evidence supporting this notion is that muscle blood volume (as measured by tHb) increased rather than decreased during cold water immersion (see Figure 3.3B).

Another possible explanation for the differences between our findings and other studies is that the water temperature in the present study (10°C) was lower than in other studies, which generally ranged from 30–44°C (Farhi & Linnarsson, 1977; Weston et al., 1987; Bonde-Petersen et al., 1992; Shiraishi et al., 2002). Participants were also only immersed in water up to their umbilicus, whereas participants in these other studies were immersed in water up to their chest or higher
Table 3.1. Peak isokinetic torque (Nm) (mean ± SD) during the fatigue task at baseline, after the cold water immersion (CWI), and after active recovery (ACT) conditions. N.B. Baseline performance was measured 7 d prior to the first trial, and was used as a ‘pre-exercise’ comparison for both experimental conditions. *Significant difference from contractions 1–5 (p < 0.05).

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(Farhi & Linnarsson, 1977; Weston et al., 1987; Bonde-Petersen et al., 1992; Shiraishi et al., 2002). Systematic comparisons on the effects of ambient temperature on changes in cardiac output and stroke volume are limited, and have produced equivocal findings. Bonde-Petersen et al (Bonde-Petersen et al., 1992) reported no significant changes in cardiac output after sitting at room temperature, 15 min immersion in water at 15.2 or 34.6°C, whereas cardiac output increased after immersion in water at 43.8°C. By contrast, stroke volume increased only after immersion in water at 15.2°C (Bonde-Petersen et al., 1992). Weston et al (Weston et al., 1987) found that cardiac output increased by 30% and stroke volume increased by 50% after 15 min immersion in water at 33–35°C, whereas they increased more substantially after immersion in water at 37–39°C. Raven et al (Raven et al., 1975) observed that cardiac output increased in a linear fashion as ambient air temperature decreased from 28 to 5°C. Stroke volume was also inversely correlated with ambient temperature \( r = -0.92; \ p < 0.01 \) (Raven et al., 1975). More research is warranted to investigate the effects of lower water temperatures (i.e., ≤15°C) on hemodynamic responses at rest and following exercise. Farhi and Linnarsson (Farhi & Linnarsson, 1977) observed that stroke volume and cardiac output increased as more of the body was immersed in water. Immersion up to the umbilicus in the present study was possibly not sufficient to increase hydrostatic pressure, stroke volume and cardiac output. In support of this notion, Muller et al (Muller et al., 2012) did not find any significant changes in stroke volume or cardiac output after immersion to the iliac crest in water at 13 or 35°C.

The rapid and sustained decline in post-exercise heart rate following cold water immersion is consistent with other studies on water immersion at rest (Farhi & Linnarsson, 1977; Gabrielsen et al., 2000; Shiraishi et al., 2002) and after endurance exercise (Stanley et al., 2012). Coupled with the rapid decrease in heart rate, we also observed that cold water immersion quickly restored heart rate variability (as measured by Ln rMSSD) after exercise. This effect is attributed to a rise in parasympathetic activation of the heart following cold water immersion (Stanley et al., 2012). Increased parasympathetic activation reduces blood pressure during the cold pressor test (Komulainen et al., 2000). Therefore, the decrease in cardiac output and stroke volume during cold water immersion in the present study may also be related to a rise in parasympathetic activation.

The decrease in cardiac output could account for the decrease in muscle blood volume that was evident in the 60 min period after cold water immersion (as indicated by changes in tHb). Other research indicates that at rest, cardiac output increases, while total
peripheral resistance decreases following immersion in water at $\geq 34^\circ$C (Weston et al., 1987; Bonde-Petersen et al., 1992; Shiraishi et al., 2002). The decrease in total peripheral resistance in these studies is indicative of vasodilation of peripheral blood vessels. However, this response seemingly contradicts the decrease in muscle and limb blood flow that others have reported after exercise and cold water immersion (Vaile et al., 2010; Ihsan et al., 2013; Mawhinney et al., 2013), and also our current findings in relation to changes in tHb. Differences in water temperature could possibly account for this disparity, although the effect of water temperature on cardiac output and total peripheral resistance remains unclear (Bonde-Petersen et al., 1992; Muller et al., 2012).

NIRS was used to examine changes in muscle oxygenation after exercise with cold water immersion and active recovery. Other research has used NIRS to investigate passive changes in tHb (as a marker of blood volume in muscle) and SmO$_2$ (as a marker of metabolic activity in muscle) in response to cold water immersion (Ihsan et al., 2013) and topical icing of muscle (Tseng et al., 2013) after exercise. A different approach was adopted in this study by measuring changes in SmO$_2$ desaturation and resaturation in response to MVCs, which is sufficient to block blood flow and thus $O_2$ delivery to muscle (Sadamoto et al., 1983). This allowed us to separate $O_2$ consumption from $O_2$ delivery (Ryan et al., 2013). The SmO$_2$ ½ DT during MVC increased following active recovery, whereas it remained unchanged or tended to decrease after cold water immersion. Conversely, SmO$_2$ ½ RT after MVC decreased after active recovery, whereas it tended to increase after cold water immersion. The MVC itself obviously occluded blood supply and $O_2$ availability, such that $O_2$ availability did not match the rate of $O_2$ consumption in the muscle during or after the contraction. However, this deficit was seemingly greater after cold water immersion compared with active recovery, possibly as a result of lower cardiac output, muscle blood volume and/or oxidative capacity. In support of this notion, other research indicates that SmO$_2$ desaturation in muscle during exercise occurs more rapidly when central blood flow is limited (Matsui et al., 1995). Similarly, SmO$_2$ resaturation in muscle after exercise occurs more slowly as a result of reduced central (Hanada et al., 2000) and peripheral (McCully et al., 1997) blood flow. The differences in SmO$_2$ may also reflect delayed activation of the muscle metaboreflex in response to cold water immersion (Ray et al., 1997). The SmO$_2$ minimum during MVCs was similar after cold water immersion and active recovery (Figure 3.4B). This indicates that cold water immersion did not alter total $O_2$ consumption during MVCs.

A decrease in muscle temperature after cold water immersion may aid muscle recovery by reducing rates of cellular metabolism (Bleakley et al., 2012). Others have
measured changes in muscle temperature in response to cold water immersion after various forms of exercise (Peiffer et al., 2009; Gregson et al., 2013; Mawhinney et al., 2013; Broatch et al., 2014; Ihsan et al., 2014). These studies report reductions in muscle temperature below post-exercise values ranging from 3.7°C (Broatch et al., 2014) to 9.8°C (Ihsan et al., 2014). In agreement with these findings, muscle temperature at a depth of 18 mm decreased by ∼9°C from post-exercise to the end of cold water immersion. When considering the results of all these studies, the magnitude of the decrease in muscle temperature following immersion in water at 8–10°C is generally dependent on muscle temperature prior to immersion. Muscle temperature decreases for longer periods of time after cold water immersion compared with topical icing (Rupp et al., 2012), which suggests that hydrostatic pressure may influence muscle temperature after exercise.

The reduction in central hemodynamics and muscle oxygenation/hemodynamics after cold water immersion did not appear to influence muscle function. Recovery of peak and mean isometric torque was more rapid after cold water immersion compared with active recovery. This was possibly as a result of lower muscle temperature and less accumulation of lactic acid in muscle during the MVCs (Edwards et al., 1972). Another possibility is that cold water immersion reduced muscle soreness after exercise, which might have allowed the participants to produce more force during the isometric contractions. Our findings contrast with other research demonstrating no significant effects of 5–20 min cold water immersion on recovery of maximal isometric torque after endurance exercise (Peiffer et al., 2009, 2010). Differences in the metabolic demands of resistance versus endurance exercise could account for this disparity.

In summary, in comparison with active recovery, cold water immersion reduced central and muscle hemodynamics and tissue temperature, whereas it helped to restore maximal isometric strength more rapidly. These findings complement and extend other research in relation to the effects of cold water immersion on skeletal muscle blood flow after exercise (Vaile et al., 2010; Ihsan et al., 2013; Mawhinney et al., 2013). Impedance cardiography and NIRS offer some advantages over other more invasive, complicated or costly methods for assessing central and peripheral hemodynamics. However, these methods are not without limitations. The main limitation of impedance cardiography is that it is only based on estimates of stroke volume, while NIRS is influenced by changes in skin blood flow (Davis et al., 2006). Nevertheless, the spatially resolved method can overcome this to a certain extent. Accepting these limitations, it is contended that these findings advance existing knowledge of the physiological effects of cold water immersion after exercise. Throughout this discussion, issues have been identified that warrant further
investigation. These include (i) more systematic comparisons of the effects of water temperature and the level of water immersion on hemodynamic responses and (ii) more detailed investigations into hyperemic responses in skeletal muscle after cold water immersion. Another outstanding issue is whether the acute physiological effects of cold water immersion after exercise confer any benefits for day-to-day recovery from exercise. A decrease in blood flow might reduce the delivery of amino acids to muscle after exercise, which is important for protein synthesis and muscle repair (Fujita et al., 2006). Alternatively, it might assist muscle recovery by decreasing the infiltration of inflammatory cells (Lee et al., 2005), or restricting cellular metabolism and secondary ischemic or enzymatic damage in skeletal muscle (Merrick et al., 1999). Future research should investigate the effects of regular cold water immersion and other related cryotherapies on adaptation to chronic exercise training.

Acknowledgements

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Chapter 4

Chapters 2 and 3 identified two distinct acute avenues of responses to cold water immersion; performance, and physiological responses. Assuming the regular repetition and accumulation of such responses, chronic regular use of cold water immersion may therefore lead to an enhanced adaptive response (acute promotion/maintenance of muscle function), or an attenuated response (accumulation of acute physiological responses). This chapter firstly, comprehensively investigates the chronic adaptive responses to resistance training with cold water immersion. Secondly, this chapter enhances the physiological responses identified in chapter 2 and 3 by examining how cold water immersion influences the acute anabolic cellular and molecular acute response to resistance exercise.

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Cold water immersion for recovery: are we freezing the benefits of exercise?

Llion A. Roberts¹², Truls Raastad³, James F. Markworth⁴, Vandre Casagrandê Figueiredo⁴, Havard Wiig, Ingrid Egner, David Cameron-Smith⁴, Anthony Shield⁵, Jeff S. Coombes¹, Jonathan M Peake²⁶

¹ The University of Queensland, School of Human Movement Studies, Brisbane, Australia
² Centre for excellence for Applied Sport Science Research, Queensland Academy of Sport, Brisbane, Australia
³ Norwegian School of Sport Sciences, Oslo, Norway
⁴ The Liggins Institute, The University of Auckland, Auckland, New Zealand
⁵ School of Exercise and Nutrition Sciences, Queensland University of Technology, Brisbane, Australia
⁶ School of Biomedical Sciences and Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Corresponding author:
Jonathan Peake
Institute of Health and Biomedical Innovation
Kelvin Grove, QLD 4059
Australia
Email: jonathan.peake@qut.edu.au
Phone: +61 7 3138 6140

Running title: Adaptations to resistance exercise and cold water immersion
ABSTRACT

Functional, morphological and molecular adaptations to cold water immersion after resistance exercise was investigated, through two separate studies. In the first study, 21 physically active young men performed 12 weeks of strength training 2 d·wk⁻¹, with either 10 min of cold water immersion (CWI) or active recovery (ACT) after each training session. Muscle strength, volume and mass were assessed, and muscle biopsies were collected from m. vastus lateralis before and after training in each group. Muscle strength and volume increased significantly more in the ACT group compared with the CWI group. Furthermore, isokinetic work and type II muscle fibre cross-sectional area increased significantly after training in the ACT group, but not the CWI group. In the second study, 9 physically active young men performed a bout of acute resistance on separate days, followed by CWI or ACT. Muscle biopsies were collected from m. vastus lateralis before exercise, 2, 24 and 48 h post-exercise. The number of NCAM⁺ (2–48 h) and PAX7⁺ (24–48 h) satellite cells increased significantly after exercise and ACT. By contrast, following exercise and CWI, NCAM⁺ satellite cells were only elevated at 48 h. NCAM⁺ satellite cell number was higher at 24 h, while the PAX7⁺ satellite cell number was higher at 24 and 48 h after exercise and ACT compared with CWI. p70S6K phosphorylation was elevated at 2 h after both exercise trials, but was higher after ACT. Protein abundance of 4E-BP1 was also elevated at 2 h after both exercise trials, but was not different between the trials. Phosphorylation of ERK 1- and 2 increased following exercise and CWI, but not ACT. rS6K phosphorylation was higher 48 h after exercise and ACT compared with CWI. Taken together, these data suggest that CWI attenuates increases in satellite cells and the activity of proteins that regulate muscle hypertrophy after resistance exercise. In the long term, these effects reduce gains in muscle strength and volume. Therefore, it may not be advisable to use CWI on a regular basis after resistance exercise.

Key words: Cryotherapy, adaptation, strength, anabolism
INTRODUCTION

Cold water immersion is popular post-exercise recovery strategy. It is generally assumed that when used on a regular basis, recovery modalities such as cold water immersion will help athletes to maintain required workloads during subsequent training sessions and reduce the risk of injury (Barnett, 2006). However, few studies have assessed whether cold water immersion influences long-term training adaptations to resistance training. Two studies indicated that cold water immersion attenuated gains in strength (Fröhlich et al., 2014) and muscle endurance capacity and brachial artery diameter (Yamane et al., 2006) after 4–5 weeks of resistance training. Conversely, another study demonstrated that cold water immersion enhanced strength gains after 5 days of resistance training (Burke et al., 2000). Considering this disparity and other limitations of these studies (e.g., short training periods, isolated muscle groups, no assessment of muscle hypertrophy), further research is warranted to evaluate the effects of cold water immersion and long-term adaptations to resistance training.

Cold water immersion reduces muscle blood flow at rest (Gregson et al., 2011) and after exercise (Vaile et al., 2011; Mawhinney et al., 2013). Because muscle protein synthesis is dependent on adequate blood supply (Fujita et al., 2006; Timmerman et al., 2010), this decrease in muscle blood flow in response to cold water immersion may have important implications for muscle metabolism during recovery from exercise. Lower blood flow in muscle in response to cold water immersion could reduce muscle protein synthesis, which might account (in part) for previous observations that cold water immersion attenuates gains in muscle strength and endurance capacity following resistance training (Yamane et al., 2006; Fröhlich et al., 2014). No research has investigated whether cold water immersion influences the activity of signal transduction pathways (e.g., mTOR, p70S6K, 4E-BP1) that regulate protein synthesis in muscle after resistance exercise (Mayhew et al., 2009).

Only two studies have investigated how cold water immersion influences muscle metabolism after exercise (Gregson et al., 2013; Ihsan et al., 2014). Several animal studies have examined the effects of applying ice to muscle strain or crush injuries. However, these studies have focused more on inflammation and oxidative stress, and have produced inconsistent findings (Lee et al., 2005; Carvalho et al., 2010; Puntel et al., 2011; Takagi et al., 2011). Considering the importance of the mTOR pathway and its downstream targets in regulating muscle hypertrophy (Bodine et al., 2001), more research is needed to determine whether cold water immersion influences these pathways during recovery from resistance exercise.
The aim of the present study was two-fold: (1) to examine the influence of regular cold water immersion on long-term functional and morphological adaptations to 3 months of resistance training, and (2) to investigate the effects of cold water immersion on acute hypertrophy-signalling pathways in skeletal muscle during recovery from an acute bout of resistance exercise. It was hypothesised that cold water immersion would attenuate long-term training adaptation by reducing the activity of key signalling proteins in skeletal muscle in the first 48 h following resistance exercise.

METHODS

**Experimental design**

Overall, the study was divided into two smaller studies. Study 1 consisted of a randomised controlled study in which 24 physically active young men volunteered to participate in a 12 week lower body strength training program. Before the training period commenced, the participants were matched for strength and lean mass, and were randomly assigned to a group that performed cold water immersion (n=11), or active recovery (n=9) within 10 min after each training session. Before the training period commenced and at least 2 d after the final training session, muscle strength and volume were measured, and muscle biopsies were collected from vastus lateralis at rest. Study 2 consisted of a randomised, cross-over study in which another group of 10 physically active young men volunteered to complete two bouts of one-leg resistance exercise on separate days, followed by cold water immersion or active recovery. Muscle biopsies were collected from vastus lateralis of one leg, before, 2, 24 and 48 h after both training sessions.

All participants had at least 12 months of experience in strength training, and were familiar with all exercise aspects of the studies. Prior to participation, all of the participants were informed about the requirements and potential risks of taking part, and then provided their informed consent. Both studies were approved by the Human Research Ethics Committee of The University of Queensland. Due to injury not associated with the study, three participants withdrew from Study 1, while one participant withdrew from Study 2. Accordingly, their data was subsequently excluded from the analysis. The characteristics of the participants in each study are described in Table 4.1.

**Strength training**

Strength training for both studies involved only the lower body, and was based around 8-, 10- and 12- repetition maximum (RM) loads, and weights corresponding to a proportion of each participant’s body mass. A breakdown of training loads, repetitions,
Table 4.1. Descriptive characteristics of participants in both experimental groups, for both studies. P values and effect size (d) denotes differences between groups for Study 1, and differences between legs for Study 2. *Percentage of total body mass. Strength indices were derived from the average of leg press, knee extension and knee flexion 1-RM. ACT, active recovery group. CWI, cold water immersion group.

<table>
<thead>
<tr>
<th></th>
<th>ACT</th>
<th>CWI</th>
<th>p</th>
<th>d</th>
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</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.3 ± 1.9</td>
<td>21.2 ± 2.2</td>
<td>.418</td>
<td>0.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.83 ± 0.1</td>
<td>1.81 ± 0.1</td>
<td>.237</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>79.2 ± 4.4</td>
<td>81.3 ± 11.6</td>
<td>.251</td>
<td>0.3</td>
</tr>
<tr>
<td>Lower body lean mass (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.0 ± 3.0</td>
<td>28.1 ± 4.1</td>
<td>.453</td>
<td>0.04</td>
</tr>
<tr>
<td>Strength indices (kg)</td>
<td>167 ± 24</td>
<td>175 ± 34</td>
<td>.263</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Study 2</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>22.1 ± 2.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 ± 0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>83.9 ± 15.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45° leg press 8 RM (kg)</td>
<td>149 ± 21</td>
<td>153 ± 25</td>
<td>.347</td>
<td>0.3</td>
</tr>
<tr>
<td>Knee extension 8 RM (kg)</td>
<td>36.1 ± 6.7</td>
<td>35.0 ± 6.4</td>
<td>.347</td>
<td>0.2</td>
</tr>
</tbody>
</table>

progression and exercise order is provided in Table 4.2. All strength training was supervised, and performed at normal room temperature.

Recovery therapies
Both studies involved the same recovery therapies. Cold water immersion was performed while seated in a hydrotherapy bath, with both legs and trunk immersed in water up to the level of the umbilicus (iCool iBody, iCool, Miami, Australia). Water was continuously circulated and maintained at 10.1 ± 0.3°C (Study 1) and 10.3 ± 0.5°C (Study 2) using a circulatory cooling unit (iCool LITE, iCool, Miami, Australia). Participants were instructed to perform active recovery at a low, self-selected intensity on a stationary cycle ergometer (Wattbike, Nottingham, United Kingdom). Mean power output during active recovery was 59.5 ± 9.4 W (Study 1) and 36.6 ± 13.8 W (Study 2). In Study 1, each participant’s mean power output was recorded after the first training session, and was replicated following subsequent training sessions. The participants minimised any re-warming following cold water immersion or cooling following active recovery not showering or bathing for at least 2 h after the recovery therapy in Study 1, and until after the 2 h muscle biopsy was collected in Study 2. Participants were allowed to towel dry and change clothing if desired following each therapy.
Table 4.2. Training session composition of both studies. Exercise order is denoted by 1–5 (study 1) and 1–4 (study 2). In both studies, repetition tempo was controlled with concentric phases of repetitions performed as quickly as possible, and eccentric and inter-repetition periods lasting approximately 1 s. In both studies, recovery time between sets was set at 1 min, and recovery between exercises was set at 2 min, leading to a total training session duration of approximately 45 min. RM; repetition maximum, PTBM; pre-training body mass, BM; body mass.

<table>
<thead>
<tr>
<th>Exercise Repetitions (in set order)</th>
<th>Repetition load</th>
<th>Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1. 45° leg press</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 1-2; (8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
<td></td>
</tr>
<tr>
<td>Wk 3-4; (8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
<td></td>
</tr>
<tr>
<td>Wk 5-6; (8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
<td></td>
</tr>
<tr>
<td>Wk 7-8; (8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
<td></td>
</tr>
<tr>
<td>Wk 9-10; (8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
<td></td>
</tr>
<tr>
<td>Wk 11-12; (8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
<td></td>
</tr>
<tr>
<td><strong>2. Knee extensions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 1-12; (12, 12, 12)</td>
<td>12 RM</td>
<td></td>
</tr>
<tr>
<td><strong>3. Knee flexions</strong></td>
<td></td>
<td></td>
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<tr>
<td>Wk 1-12; (12, 12, 12)</td>
<td>12 RM</td>
<td></td>
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<tr>
<td><strong>4. Walking lunges</strong></td>
<td></td>
<td></td>
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<tr>
<td>Wk 1, 4, 7, 10; (10, 12, 14)</td>
<td>Wks 1–3; PTBM+20%</td>
<td></td>
</tr>
<tr>
<td>Wk 2, 5, 8, 11; (12, 14, 16)</td>
<td>Wks 4–6; PTBM+20%+2.5 kg</td>
<td></td>
</tr>
<tr>
<td>Wk 3, 6, 9, 12; (14, 16, 18)</td>
<td>Wks 7–9; PTBM+20%+5 kg</td>
<td></td>
</tr>
<tr>
<td>Wk 1-3; (12, 12, 12)</td>
<td>BM</td>
<td></td>
</tr>
<tr>
<td><strong>5. Plyometrics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Wk 4-6; (12, 12, 12)</td>
<td>BM+50% of lunge load</td>
<td></td>
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<tr>
<td>Wk 7-9; (12, 12, 12 each leg)</td>
<td>BM+50% of lunge load</td>
<td></td>
</tr>
<tr>
<td>Wk 10-12; (12, 12, 12)</td>
<td>BM+50% of lunge load</td>
<td></td>
</tr>
<tr>
<td><strong>Study 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1. 45° leg press</strong></td>
<td>(8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
</tr>
<tr>
<td><strong>2. Single leg squat</strong></td>
<td>(12, 12, 12)</td>
<td>12 RM</td>
</tr>
<tr>
<td><strong>3. Knee extensions</strong></td>
<td>(8, 8, 10, 12, 10, 10)</td>
<td>8 RM, 10 RM, 12 RM</td>
</tr>
<tr>
<td><strong>4. Walking lunges</strong></td>
<td>(12, 12, 12)</td>
<td>12 RM</td>
</tr>
</tbody>
</table>

**Muscle function assessment**

For Study 1, familiarisation was performed, and pre-training muscle strength for training load prescription was assessed over two days, 10–14 days prior to the first training session. On day 1, unilateral isometric torque, isokinetic work, and rate of force development were assessed. On the second day, bi-lateral isotonic (1, 8, 10 and 12-RM) leg press strength, bi-lateral 1 and 12-RM knee flexion and extension strength, and unilateral peak isometric torque were measured. Walking lunges and plyometric jumps were also performed for further familiarisation. Post-training strength and muscle function
were assessed in the 12th week of training, in place of the last (i.e., 24th) training session. This testing was performed 48 h following the last training session to avoid any residual fatigue from prior training.

Isometric and isokinetic strength, and rate of force development were measured using the dominant leg on an isokinetic dynamometer (Cybex 6000, CSMI, Stoughton, MA, USA). To perform these measurements, the lateral condyle of the femur was aligned with the axis of rotation, and the seat angle was fixed at 90°. Peak isometric knee extension strength was identified as the peak torque measure at any time during two 5 s contractions, separated by 90 s, and performed at 70° knee angle (full knee extension = 0°). Isometric rate of force development was assessed over the initial 500 ms of the contraction. Isokinetic work was assessed by the completion of an isokinetic task consisting of 50 isokinetic knee extensions performed over a 90° range, at a velocity of 90°·sec⁻¹. Repetition tempo was set at 0.5 Hz by an audio signal, and knee flexion velocity was set at 250°/sec to allow passive flexion following each knee extension.

All data were collected at 1,000 Hz using a custom-designed Labview script (Labview, National Instruments Corp., Texas, USA), and stored on a personal computer for offline analysis. The test re-test coefficient of variation for peak torque from the 5 s isometric contraction and work performed over the isokinetic task was 2.1% and 3.5%, respectively.

For Study 2, unilateral knee extension and leg press 8, 10 and 12-RM strength for both legs was assessed on one day, 4–5 days prior to each experimental trial. Single-leg squats and walking lunges were also performed for further familiarisation, and to reinforce correct technique.

Muscle mass assessment

Muscle mass was assessed in Study 1 by measuring the volume of the quadriceps muscle group of the dominant leg using MRI (Magnetom Sonata 1.5T, Siemens AG, Munich, Germany), and also by measuring the thigh and gluteal muscle mass of both legs using DXA (DXA, Hologic Delphi, Hologic Inc., Bedford, USA). MRI scans were de-identified for blind assessment. Quadriceps volume was calculated between the most proximal and distal slices in which the vastus lateralis was visible. Volume was assessed using semi-automated manual segmentation and open-source software (Yushkevich et al., 2006), and then converted to lean mass based on a muscle density of 1.04 kg·L⁻¹ (Vierdort, 1906). The coefficient of variation for quadriceps muscle volume was 2.8%
based on calculations from six pre-training and six post-training MRI scans. Thigh and gluteal muscle mass was measured using native software (Hologic Delphi, Hologic Inc., Bedford, USA), from a region of interest between the iliac crest and the distal femur on each leg. The coefficient of variation for thigh and gluteal muscle volume was 0.7% based on calculations from six pre-training and six post-training DXA scans. Pre-training measurements were performed at rest, 8–10 days prior to the first training session, and post-training at rest over days 4 and 5 after the final training session.

**Muscle tissue collection**

Muscle biopsies for both studies were collected from the mid portion of the *vastus lateralis* muscle. Biopsies for Study 1 were collected following an overnight fast, from the dominant leg. Post-training biopsies were collected ~3 cm proximal to the pre-training site. Pre-training biopsies were collected over 4–5 d before the first training session, while post-training biopsies were collected 6–7 d after the last training session. Biopsies from Study 2 were collected in the fed state before exercise and again at 2, 24 h and 48 h after each exercise trial. Pre-exercise and 2 h post-exercise biopsies were collected from the same incision. However, the pre-exercise biopsy was collected with the needle inserted in a distal direction, whereas the 2 h biopsy was collected with the needle inserted in a proximal direction. This method ensured that the two biopsy sites were separated by at least 3 cm. Biopsies at 24 and 48 h were collected from separate incisions, each ~3 cm proximal from the previous. All biopsies were collected under local anaesthesia (10 mg/ml Xylocaine) with a 6 mm Bergström needle (Pelomi, Alberteslund, Denmark), modified for the application of manual suction. All muscle tissue was quickly washed in sodium chloride, and any fat, connective tissue or blood was dissected prior to weighing and freezing in liquid nitrogen (for western blotting) or isopentane cooled in dry-ice (for immunohistochemistry). All samples were stored at −80°C until analysis.

**Control procedures**

For Study 1, the participants were provided with protein and carbohydrate before and after each training session in an attempt to control for the degree of aminoacidemia experienced. The participants were given a 30 g serve of whey protein isolate (BSc WPI, Gold Coast, Australia) to drink 60 min before each training session, and following the completion of the recovery therapy. They were also given a recovery bar (BSc Missile Performance Energy Bar, Gold Coast, Australia) containing 18 g protein and 30.7 g
carbohydrate to eat 2 h after each training session. For Study 2, prior to each trial, the participants ate the same meal 2 h prior to the pre-exercise muscle biopsy, and were then given a 30 g serve of the same whey protein isolate to drink prior to the recovery therapy. The participants then fasted until the 2 h biopsy was taken, after which they were provided with another 30 g of whey protein isolate to drink after the biopsy.

The participants were instructed to avoid consuming any additional supplements for the duration of Study 1, and from 96 hrs before each pre-exercise biopsy until the 48 h muscle biopsy of Study 2. They consumed a daily intake of 2 g·kg\(^{-1}\) protein from their diet throughout the training period of Study 1, and from 48 h before each experimental trial until the 48 h muscle biopsy of Study 2. Dietary intake during Study 1 was monitored through a 5 day food diary completed every third week. Dietary intake encompassing the first experimental trial of Study 2 was recorded in a food diary, and replicated for the second experimental trial.

The participants refrained from any additional lower body resistance exercise during the training period of Study 1, for 24 h prior to any assessment of muscle function and muscle mass. They also avoided additional exercise for 48 h prior to all biopsies.

**Biochemical analysis**

**Western blotting**

Approximately 25 mg of muscle tissue (dry weight) was homogenised on ice in 15 µl·mg\(^{-1}\) of 1× RIPA lysis buffer (Millipore, #20-188) with added protease and phosphatase inhibitors (Halt\(^{\text{TM}}\), Thermo Scientific, #78442). Samples were centrifuged at 15,000 g for 10 min at 4°C before the supernatant was collected and analysed for protein concentration by bicinchoninic acid (Pierce\(^{\circledR}\) BCA Protein Assay Kit, # 23225). Working samples were diluted to 2 µg·µl\(^{-1}\) of protein in distilled water and Laemmlli loading buffer, and then heated at 95°C for 5 min. Experimental samples and a pooled control (10 µl) were loaded on to 8–15% acrylamide self-cast gels for separation by electrophoresis. Proteins were transferred to polyvinylidene fluoride membranes by semi-dry transfer (Trans-Blot\(^{\circledR}\) Turbo\(^{\text{TM}}\), Bip-rad, Hercules, USA) and transfer packs (Bio-rad, Hercules, USA, #170-4157) prior to blocking for 2 h at room temperature in 5% bovine serum albumin in tris-buffered saline with 1% tween (TBST). Membranes were incubated overnight at 4 °C with primary antibodies against phosphorylated (p) p70S6 kinase\(^{\text{Thr421/Ser424}}\) (1:10,000; #9204), p-4E-BP1\(^{\text{Thr37/46}}\) (1:10,000; #9459), p-ERK1/2\(^{\text{Thr202/Tyr204}}\) (1:10,000; #4377), p-rpS6\(^{\text{Ser235/236}}\) (1:10,000; Abcam #12864) and total (T) proteins for T-p70S6 kinase (1:10,000; #2708), T-
ERK1/2 (1:10,000; #4695) and T-rpS6 (1:10,000; Abcam #40820). All antibodies were obtained from Cell Signaling (Danvers, MA), unless stated otherwise. Membranes were thereafter washed in TBST, incubated with secondary antibody at room temperature for 1 h, and washed in TBST again. Immunoreactive bands were detected by chemiluminescence (Amersham™ ECL Select™, GE Healthcare, #RPN2235) on a ChemiDoc XRS+ imaging system (Bio-rad, Hercules, USA). Densiometry was conducted using native software (ImageLab V4.1, Bio-rad, Hercules, USA). Bands were generally expressed relative to the pooled control before they were adjusted for expression of the housekeeping protein GAPDH (1:10,000; Abcam, #9485) to control for equal protein loading. phospho-4EBP1 was related to the pooled control and adjusted for the expression of the γ-form of 4-EBP1 (Kimball et al., 1997; Vary, 2006).

**Immunohistochemistry**

Muscle cross-sections (8 μm thickness) were cut at −20 °C on a cryostat (CM3050 S, Leica, Germany), mounted on microscope slides and air-dried at room temperature. Sections for the identification of fibre cross-sectional area were blocked for 60 min in 1% goat serum albumin with phosphate-buffered saline with tween (1%) before incubation overnight at 4 °C in primary antibodies against type I myosin (1:1,000; DSHB, #BA-F8) and type II myosin (1:1,000; DSHB, #SC-71). Sections were then incubated at room temperature for 60 min with respective secondary antibodies (Alexa Fluor® A-11005 or A-11001, Invitrogen, USA). Microscope slides were covered with a coverslip, sealed and stored at room temperature. Muscle sections were visualised using a high-resolution camera (CoolCube 1c, Meta-Systems, MA, USA) mounted on a fluorescence microscope and equipped with the ISIS fluorescence imaging system (MetaSystems, Altlussheim, Germany).

Sections for the satellite cell counts were incubated with primary antibodies against NCAM and PAX7 (1:200; Abcam, #AB9018 and #AB55494) before incubation with goat anti-mouse secondary antibody (Alexa Fluor® 488, Invitrogen, USA). Finally, nuclei were stained on sections with DAPI (ProLong Gold Antifade Reagent with DAPI, P36935, Invitrogen, USA). Slides were covered with cover slips and stored at room temperature prior to visualisation with an Axi-ocam camera (Zeiss, Oberkochen, Germany) mounted on an Axioskop-2 light microscope (Zeiss). Satellite cells were identified by DAPI staining surrounded by NCAM or PAX7, localised between the sarcolemma and basal lamina (Hanssen et al., 2013).
Only regions of interest were analysed from sections. Any damaged or transverse orientated fibres were excluded. A mean ± SD of 440 ± 256 fibres were analysed in samples from Study 1, whereas a mean ± SD of 296 ± 136 fibres were analysed in samples from Study 2. Images for fibre type and cross sectional area analysis were exported to a personal computer, and analysed in a blinded manner by open-source software (ImageJ; Schneider et al., 2012). Satellite cells were visualised and counted at ×40 magnification, and are presented as the number of positive cells per fibre.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences program (V.19, IBM, New York, USA). All data were initially assessed for normal distribution by Shapiro-Wilk analysis. A two-factor repeated measures ANOVA was used to evaluate time effects and trial × time interaction effects. Relative differences (%) between groups (change from pre- to post-training) were evaluated by unpaired t-tests or Mann Whitney-U test, dependent upon normality. The false discovery rate was used for multiple comparisons of time points within and between trials. Cohen’s effect size (d) was calculated to compare the magnitude of changes over time and between conditions, and assessed as follows: 0.2 to ≤ 0.5 = small effect, 0.51 to ≤ 0.8 = moderate effect, and ≥ 0.8 = large effect. Significance was set at a level of p < 0.05.

RESULTS

Performance responses following strength training

Maximal strength (i.e., 1-RM) during leg press (64 ± 27% vs 43 ± 12%) and knee extension (39 ± 9% vs 20 ± 9%) increased significantly after training for active recovery and cold water immersion groups, respectively (p < 0.001; F = 134 to 273; see Figure 4.1). However, gains in strength were influenced by group (time*group interaction p = 0.018 to 0.022, F = 6.78 to 10.83) and were significantly lower in the cold water immersion group compared with the active recovery group (leg press p = 0.033, d = 1.0; knee extension p < 0.001, d = 3.0). Maximal isometric torque (25 ± 12%) and rate of force development (90 ± 77%) were influenced by the recovery condition (time*group interaction p = 0.014, F = 7.385), with isometric torque only increasing significantly following training in active recovery group (p < 0.001; see Figure 4.2). These responses were significantly greater compared with cold water immersion (p < 0.001; isometric torque d 1.2, rate of force
development $d$ 1.5). Similarly, isokinetic work performed over contractions 1–25 was influenced by training and the recovery therapy (time*group interaction $p = 5.201$) increased significantly after training in the active recovery group ($p = 0.032; 19 \pm 11\%; d$ 1.5), but not the cold water immersion group ($p = 0.525$). Training did not alter maximal isokinetic torque or the total isokinetic work performed during contractions 26–50 by either group (all $p > 0.05$; see Figure 4.2).
Muscle mass accretion following strength training

Quadriceps muscle mass increased significantly \((p \leq 0.001)\) following training in both the active recovery group \((11.4 \pm 3.5\%)\) and cold water immersion group \((3.7 \pm 2.3\%)\) (Figure 4.3A). Quadriceps muscle mass accretion was significantly smaller in the cold water immersion group compared with the active recovery group \((p < 0.001; d 3.7)\). Thigh and gluteal muscle mass also increased significantly \((p < 0.001)\) following training in the active recovery group \((5.6 \pm 1.9 \%)\) and cold water immersion \((3.9 \pm 2.3 \%)\) group (Figure 4.3B). In contrast with quadriceps muscle mass, there was no statistically significant difference \((p = 0.161)\) in thigh and gluteal muscle mass between the groups after training, despite a large effect size of 1.1 in favour of the active recovery group.
Figure 4.3. Mean ± SD change in the percentage change in quadriceps muscle mass (A) and thigh and gluteal muscle mass (B) following training. ** $p < 0.001$ change from Pre. # Significant difference between groups ($p < 0.05$). $d$ values displayed for comparisons of relative differences between groups.

Type II fibre cross-sectional area increased following training in the active recovery group ($p = 0.009$; 17.1 ± 5.1 %), whereas there was no increase after training in the cold water immersion group ($p < 0.05$) (Figure 4.4A). No significant difference ($p = 0.657$) existed between the groups post-training, despite a moderate effect size of 0.6 in favour of the active recovery group. There were no differences in type II muscle fibre-specific, and total myonuclei counts before or after training ($p > 0.05$), however counts were influenced by training ($p = < 0.001$ to 0.002; $F = 14.538$ to $22.325$). Type II ($p = 0.001$; $d = 1.7$) and total counts ($p = 0.008$; $d = 1.3$) only increased following active recovery. No increase existed following cold water immersion (all $p > 0.05$).
Anabolic responses to acute resistance exercise

Phosphorylation of p70S6K$^{Thr421/Ser424}$ was greater than pre-exercise at 2 h following the active recovery trial ($p = 0.002; 3.6$-fold) and the cold water immersion trial ($p = 0.002; 2.2$ fold) (Figure 4.5A). Following active recovery, phosphorylation remained elevated above pre-exercise at 24 h ($p = 0.007; 2$-fold), and tended to remain elevated at 48 h ($p = 0.068; 1.8$-fold). Phosphorylation was greater following active recovery compared with cold water immersion at 2 h ($p = 0.048; 0.9$-fold; $d = 4.7$) and 24 h ($p = 0.049; 0.6$-fold; $d = 5.3$). Phosphorylation of p70S6K$^{Thr389}$ was increased 2 h following exercise in both active recovery ($p = 0.008; 1.7$-fold) and cold water immersion trials ($p = 0.024; 1.6$-fold). Phosphorylation remained elevated at 24 and 48 h following active recovery ($p = 0.020$ to 0.032; 1.3 to 1.9-fold), whereas phosphorylation was not elevated at either time point following cold water immersion ($p > 0.05$) (Figure 4.5B). Following active recovery, phosphorylation was accompanied by an increase in protein content at 48 h ($p = 0.030$;
1.3 fold) (Figure 4.5C). p70S6K total protein tended to be higher at 2 h ($p = 0.1; 0.3\text{-fold}; d 1.0$) and 24 h ($p = 0.12; 0.2\text{-fold}; d 0.8$) after active recovery. At 48 h after recovery, p70S6K total protein remained elevated ($p = 0.030; 0.3\text{-fold}; d 0.4$), and was higher than it was after cold water immersion ($p = 0.021; 0.3\text{-fold}; d 1.8$). p70S6K total protein remained unchanged following cold water immersion point ($p \geq 0.3$).

Hyperphosphorylation of 4E-BP1 (assessed by mobility shift: appearance of g isoform) increased above pre-exercise at 2 h following active recovery ($p = 0.008; 4.2\%; d 4.1$) and cold water immersion ($p = 0.008; 4.1\%; d 5.9$) (Figure 4.6). The phosphorylation state of 4E-BP1 had returned to pre-exercise values by 24 after both trials. There were no statistically significant differences in the expression of 4E-BP1 between the trials at any time point.

Phosphorylation of ERK-1$^{Thr202/Tyr204}$ and ERK-2$^{Thr185/Tyr187}$ tended to increase after cold water immersion ($p = 0.067$) (Figures 4.7A-B). Phosphorylation of ERK-1 and -2 tended to remain elevated at 24 h following cold water immersion (ERK-1 $p = 0.057; 4.5\text{-fold}$, and ERK-2 $p = 0.091; 3.6\text{-fold}$). Phosphorylation of ERK-1 or -2 did not increase above pre-exercise following the active recovery trial ($p \geq 0.1$), and there were no differences between the trials at any time ($p \geq 0.1$). Furthermore, ERK-1 and 2 total protein did not change over time or differ between conditions ($p > 0.05$).
Figure 4.5. Mean ± SD fold change in phosphorylated p70S6K^{Thr421/Ser424} (A), phosphorylated p70S6K^{Thr389} (B) and total p70S6K (C). * $p < 0.05$ change from PRE, # $p < 0.05$ difference between groups.
Phosphorylation of rpS6$^{\text{Ser240/244}}$ and rpS6$^{\text{Ser235/236}}$ did not change after exercise in either trial ($p > 0.05$; figures 4.8A and B). However, protein content of rpS6 differed between conditions, and was influenced by the recovery therapy (condition $p = 0.025$; interaction $p = 0.012$). Protein content was increased 48 h following active recovery ($p = 0.024$; 0.43 fold; $d$ 1.9), whereas it remained unchanged following cold water immersion. rpS6 total protein was greater at 24 h ($p = 0.027$; 0.4 fold; $d$ 1.2) and 48 h ($p = 0.032$; 0.5 fold; $d$ 1.5) following active recovery compared with cold water immersion (Figure 4.8C).

**Figure 4.6.** Mean ± SD % change in γ-form of 4E-BP1. * $p < 0.05$ change from PRE.

The number of PAX7$^+$ cells increased above pre-exercise at 24 h ($p = 0.023$; 20%; $d$ 1.9) and 48 h ($p = 0.004$; 50%; $d$ 2.7) following the active recovery trial (Figure 4.9A). No increase in PAX7$^+$ satellite cells existed following cold water immersion at any time ($p = 0.155$ to 0.786). PAX7$^+$ cell count was greater following active recovery compared with cold water immersion at 48 h ($p = 0.013$; 30%; $d$ 1.4). The number of NCAM$^+$ cells increased above pre-exercise values at all time points following the active recovery trial ($p = 0.012$ to 0.031; 1.1% to 1.3%; $d$ 1.9 to 2.4) (Figure 4.9B). No increase in NCAM$^+$ cells was apparent following cold water immersion until 48 h ($p = 0.014$; 10%; $d$ 2.1). The number NCAM$^+$ cells was significantly higher 24 h after active recovery compared with cold water immersion ($p = 0.024$; 0.2%; $d$ 1.7).
DISCUSSION

The aims of the present investigation were to examine the influence of regular cold water immersion on chronic adaptations to strength training, and to investigate the effects of cold water immersion on acute hypertrophy-signalling pathways and satellite cell kinetics in skeletal muscle after acute strength exercise. The key findings were: 1) regular cold water immersion attenuated increases in muscle strength, muscle mass, fibre cross-sectional area and myonuclei accretion following 3 months of strength training; and 2) cold water immersion...
Figure 4.8. Mean ± SD fold change in rpS6<sup>Ser240/244</sup> phosphorylation (A), rpS6<sup>Ser235/236</sup> phosphorylation (B) and total protein (C). # p < 0.05 difference between conditions.
immersion reduced and/or delayed the activation of satellite cells and kinases that regulate muscle protein synthesis after strength. Collectively, these findings suggest that diminished long-term gains in muscle strength and hypertrophy may have resulted from the cumulative suppression of anabolic signalling and satellite cell activity in response to cold water immersion after each training session. Accordingly, it may not be advisable for athletes to use cold water immersion after every training session, particularly during periods of training that are intended to induce adaptations such as increasing muscle strength and mass.

The present findings build on the findings of the three studies that have previously investigated the effects of cold water immersion on adaptation to training. Yamane et al. (2006) investigated changes in muscle endurance capacity and muscle strength after 4 weeks of handgrip training (3× per week) with cold water immersion (20 min at 10 ± 1°C) or passive recovery. Cold water immersion did not negatively affect performance adaptation, however blunted any increase in artery diameter. More recently, Fröhlich et al. (2014) evaluated changes in strength after 5 weeks of leg curl exercise (frequency not specified) with cold water immersion (3 × 4 min at 12 ± 1.5°C) or passive recovery. Improvements in hamstring strength were significantly smaller in the leg that was treated with cold water immersion. In contrast with the findings from the present study, and these other two studies (Yamane et al., 2006; Fröhlich et al., 2014), Burke et al reported a greater increase in isometric hip flexor strength after 5 d of isometric training combined with cold water immersion (10 min at 8 ± 1°C) compared with passive recovery after each session. No previous study has investigated the effect of cold water immersion on the muscle hypertrophy response after strength exercise. In the present study, we addressed some of the limitations of these studies by including a longer training period, training multiple muscle groups, evaluating a wide array of muscle functions, and assessing changes in muscle mass and myofibre cross-sectional area. The results of this present study therefore provide stronger and more comprehensive evidence for the attenuation of training adaptation in response to cold water immersion.

To determine the potential mechanisms by which cold water immersion attenuated gains in muscle strength and hypertrophy, we conducted a follow-up study in which we examined the effects of cold water immersion on signal transduction pathways that regulate muscle protein synthesis after strength exercise. We focused on changes in downstream targets of mTOR and ERK pathways, including p70S6K, 4E-BP1 and rpS6, which all regulate muscle protein synthesis after exercise (Mayhew et al., 2009). p70S6K phosphorylation at Thr421/Ser424 increased significantly at 2 h and 24 h after exercise in
the active recovery trial. By contrast, p70S6K phosphorylation increased only at 2 h after exercise in the cold water immersion trial, and the level of activation was lower compared with the active recovery trial. 4E-BP1 activation increased at 2 h after exercise in both the active recovery and cold water immersion trials, with no difference between the trials. rpS6 phosphorylation (Ser240/244) did not change significantly after exercise in either trial, although it was higher 48 h after exercise in the active recovery trial compared with the cold water immersion trial. We also observed with two distinct satellite cell markers (PAX7 and NCAM), that the normal increase in muscle satellite cell number occurring in the latter stages of post-exercise recovery was inhibited in the cold water immersion trial. The number of PAX7$^+$ cells beneath the basal lamina increased only after exercise in the active recovery trial. Furthermore, the number of NCAM$^+$ cells increased at 2, 24 and 48 h after exercise in the active recovery trial, whereas these cells only increased at 48 h after exercise in the cold water immersion trial.

Figure 4.9. Mean ± SD fold change in PAX7 (A) and NCAM (B) positive satellite cells. * $p < 0.05$ change from PRE. # $p < 0.05$ difference between groups.
exercise in the cold water immersion trial. If cold water immersion blunts the activation of p70S6K and satellite cells after individual training sessions, and this occurs repeatedly after multiple training sessions, then this could diminish long-term gains in muscle hypertrophy and strength, (Baar & Esser, 1999; Terzis et al., 2008) as we observed in the training study.

Only two other studies have investigated how cold water immersion influences muscle metabolism in humans after exercise. Ihsan et al (2014) reported that 10 min of immersion in cold water (10°C) increased mRNA expression of PGC-1α (but not VEGF or nNOS) 3 h after endurance exercise. By contrast, Gregson et al (2013) observed that 10 min of immersion in cold water (8°C) did not alter muscle glycogen resynthesis following endurance exercise. Several animal studies have demonstrated that icing after muscle injury reduces markers of inflammation and oxidative stress in muscle (Hurme et al., 1993; Lee et al., 2005; Carvalho et al., 2010; Puntel et al., 2011; Takagi et al., 2011). However, in partial support of our findings, Takagi et al (2013) found that icing attenuated muscle fibre regeneration and caused greater fibrosis 4 weeks after muscle crush injury. They attributed this maladaptation to a delay in macrophage infiltration, satellite cell activity and gene expression of essential growth factors such as TGF-β1 and IGF-1 (Takagi et al., 2011). In another animal study, Fu et al (Fu et al., 1997) demonstrated that regular cold water immersion actually caused more rapid and severe muscle damage during long-term endurance training. They suggested that cold water immersion may have masked muscle soreness and reduced swelling after exercise, which may have allowed the rats to exercise more intensely the following day.

The physiological and/or biochemical factors responsible for the decrease in the activation of p70S6K and satellite cells after cold water immersion are not immediately obvious. But reductions in muscle blood flow and temperature may be involved. Fujita et al (2006) reported that muscle protein synthesis is strongly correlated with muscle blood flow ($r = 0.79$, $p <0.0001$). Furthermore, Timmerman et al (2010) demonstrated that muscle protein synthesis increases in response to stimulation of muscle blood flow. Cold water immersion reduces blood flow to muscle (Gregson et al., 2011; Mawhinney et al., 2013) and the limbs (Vaile et al., 2011). By reducing muscle blood flow, cold water immersion may reduce the delivery of amino acids to skeletal muscle, and this could result in less activation of signalling pathways that control muscle protein synthesis after exercise. The timing of alterations in blood flow relative to exercise may be a critical factor regulating muscle protein synthesis. In contrast to the present findings, restricting blood flow during exercise stimulates the expression of p70S6K and rpS6 after exercise (Fry et al., 2010).
Cold water immersion also reduces muscle temperature (Gregson et al., 2011; Gregson et al., 2013; Mawhinney et al., 2013), and this might influence the expression of genes or the activity of transcription factors that regulate muscle growth. For example, cold shock inhibits myoblasts from expressing myogenin and forming myotubes (Shima & Matsuda, 2008), which might partially reduce muscle hypertrophy. Cold shock also increases the expression of the cell cycle regulatory proteins p53 and p21 in various cell types (Matijasevic et al., 1998; Roobol et al., 2009). These proteins play an important role in regulating muscle growth and remodelling (Chen et al., 2002; Fox et al., 2014). Changes in their expression/activity in response to cold may therefore influence muscle hypertrophy. Cold shock induces the expression of other proteins such as RNA-binding motif protein-3 (RBM3) in muscle cells, which promotes cell survival and prevents apoptosis (Ferry et al., 2011). It therefore seems unlikely that changes in RBM3 can account for the diminished muscle hypertrophy following strength training and cold water immersion.

Cold water immersion strongly activated ERK1/2 2 h after strength exercise. However, because ERK pathway is rapidly activated after exercise (Creer et al., 2005; Galpin et al., 2012), it is possible ERK activation peaked less than 2 h post-exercise in the active recovery trial. The reduced phosphorylation of p70S6K at Thr421/Ser424 and the increased activation of ERK1/2 at 2 h post exercise suggest cold water immersion delayed activation of the ERK pathway. ERK1/2 appears to play a varied, biphasic role in myogenic cells. Specifically, ERK1/2 inhibits myoblast proliferation, whereas it promotes myoblast differentiation (Feng et al., 2013). Therefore, activation of ERK1/2 after cold water immersion in the early recovery period after strength exercise may have attenuated the proliferation of myoblasts. In turn, this could have contributed to the smaller gains in muscle mass after strength training and cold water immersion.

Conclusions and perspectives

In conclusion, these two studies offer novel and important insights into how cold water immersion during recovery from strength exercise affects chronic training adaptations and some of the molecular mechanisms that underpin such adaptations. Cold water immersion appeared to attenuate activation of p70S6K in the first 2 h after strength exercise and delay or inhibit the activation of satellite cells 24–48 h later. Furthermore, the increase in ERK1/2 activation in response to cold water immersion may have inhibited myogenic differentiation during a period of muscle protein synthesis soon after strength exercise. These effects may have compounded over time to diminish increments in muscle mass and strength. The participants in this study were only recreationally active, and not
elite athletes. Therefore, the findings of this study may not be entirely applicable to elite athletes. Nevertheless, from a practical perspective, the results of this study challenge the notion that athletes should use cold water immersion after every training session. A more suitable approach may be to avoid cold water immersion (or use it sparingly) during phases of the training season that are intended to promote the greatest gains in muscle mass and strength.

Conflict of interest
None

Author contributions
L.A.R., A.S., D.C.S., T.R., J.C., and J.P. designed and conceived the studies. L.A.R. and J.M. performed the analysis of biological samples. L.A.R., and J.P. wrote the manuscript. All authors helped interpret the data, and all authors approved the final version of the manuscript.

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Chapter 5

Conclusions
5.1 Summary of findings

The overall objective of this thesis was to investigate the efficacy of using cold water immersion as a recovery therapy following resistance exercise. To fulfill this objective, the underlying aims of the research were two-fold: (1) to explore the cellular, molecular and physiological effects of post-resistance exercise cold water immersion, and (2) to investigate how these physiological responses influence muscle function. These aims were addressed through four separate studies. Study 1 (chapter 2) examined the effects of cold water immersion on recovery of maximal and submaximal muscle function, muscle temperature, soreness and swelling, blood gases, endothelin-1, myoglobin and IL-6 in the first few hours after bi-lateral, high-intensity resistance exercise. Study 2 (chapter 3) examined the effects of cold water immersion on muscle strength, central and peripheral haemodynamics, heart rate variability and muscle temperature in the hour after unilateral resistance exercise. Studies 3 and 4 (chapter 4) examined the effects of regular cold water immersion on functional and morphological adaptations to chronic resistance training, and on anabolic cellular and molecular responses in muscle after acute resistance exercise.

The first study was designed to replicate the demands of exercising with a large muscle volume twice within a short space of time, such as training or competitive environments, where the amount of submaximal work that is performed is crucial. Therefore, a high-intensity bi-lateral, lower body resistance training session was adopted, along with the assessment of maximal (isometric squat, weighted- and un-weighted squat jump or countermovement jump) and sub-maximal muscle function (6 sets of 10 squats). To control the resistance exercise bout preceding the immersion period more precisely, and to measure muscle function, a unilateral exercise model on a dynamometer was adopted for the second investigation. Maximal (isometric knee extensor torque), and sub-maximal muscle function (work completed over 50 x isokinetic knee extensions) was assessed once more. Cold water immersion did not completely counteract the decrease in maximal muscle function that occurred after resistance exercise in both studies. However, compared with active recovery, it did help to restore maximal muscle function in the second study. Cold water immersion also enhanced recovery of sub-maximal muscle function 6 h post-exercise in the first study. Despite the difference in the type of resistance exercise (bi-lateral vs unilateral) between the two studies, the immersion protocol (10 min at 10°C) was the same. The only difference was immersion depth. Immersion depth was to the clavicle in the first study, whereas it was only to the umbilicus in the second
investigation (to allow the attachment of measurement apparatus to the torso). Cold water immersion may therefore be effective between resistance exercise bouts in competitive or training environments, where recovery from a training session in preparation for the next event or training session is vital.

Figure 5.1 provides a potential mechanistic framework to explain the changes in muscle function after cold water immersion. The decrease in muscle temperature observed in studies 1 and 2 could have underpinned muscle function responses in both studies. A delay in the activation of the muscle metaboreflex (resulting in less lactic acid production; Ray et al. 1997), and an increase in the recruitment of type II muscle fibres (as a result of lower muscle oxygenation; Moriati et al. 1992; Yasuda et al. 2010) could account for the faster restoration of muscle function after cold water immersion. However, as muscle temperature only remained below pre-exercise temperatures for ~35 min in both studies, other physiological factors were likely involved, in particular in the following hours. Hydrostatic pressure induced by immersion is known to reduce oedema and swelling (Wilcock et al., 2006). Furthermore, nerve conduction velocity (lower perception/higher threshold for pain) is known to decrease in response to cooling (Algafly & George, 2007). The decrease in the perception of muscle soreness combined with decreases limb volume could positively influence subsequent performance (see response from 4–6 h in study 1).

The contribution of cardiovascular responses to performance cannot be overlooked. Cold water immersion is known to reduce peripheral macrovascular and microvascular blood flow/volume (Ihsan et al., 2013; Mawhinney et al., 2013). Results from study 2, enhance this knowledge by providing data on reduced cardiac output and microvascular blood volume following immersion, together with quicker recovery of heart rate variability. A reduction in local blood flow in muscle may firstly reduce primary and secondary muscle damage (eluded to be decreased myoglobin efflux in study 2). Similarly, a reduction and/or delay in lactate concentrations observed in study 1 could be a reflection of reduced clearance compared with active recovery; in particular, considering post-exercise low intensity exercise (such as active recovery) is known to increase lactate clearance from muscle due to the maintenance of blood flow (Ahmaid et al., 1996).

Systemic and peripheral reductions in blood flow in both investigations may also account for the observed oxygenation responses. The systemic reduction in venous blood $O_2$ saturation identified in the first study, could well underpin the delay in skeletal muscle
Figure 5.1. Spider diagram of known (solid arrows) and postulated (dashed arrows) interactions between cold water immersion, physiological mechanisms, and effects upon acute and chronic muscle function. Coloured shapes indicate factors that were measured in this thesis.
oxygenation (SmO$_2$) observed following contractions in the second study. A longer recovery time for SmO$_2$ after muscle contractions indicates that SmO$_2$ demand exceeds SmO$_2$ supply. Cold water immersion may decrease blood temperature together with tissue and core temperatures. Therefore, considering that the haemoglobin dissociation curve is temperature-sensitive (Bacher, 2005), a reduction in systemic blood temperature may account for the reduced O$_2$ saturation.

Changes in systemic and local oxygenation status may also play a role in augmenting the metabolic state within the muscle. As IL-6 is released from muscle in response to glycogenolysis (Keller et al., 2001), the increase in plasma IL-6 concentration after cold water immersion in study 1 may reflect this response. However, IL-6 also plays a pro- and anti-inflammatory role (Paulsen et al., 2012). Because IL-6 was measured systemically (from venous blood), neither the origin of the measured IL-6, or its role in a pro- or anti-inflammatory response can be identified.

Although some of these physiological and biochemical responses might benefit short-term recovery of muscle function after resistance exercise, they may play a different role in long-term adaptation to resistance training. Once more, temperature and blood flow responses following immersion are likely to be important factors influencing training adaptations. Some anabolic responses, such as mTOR kinase activity are influenced by amino acid delivery, and are sensitised to this delivery post-exercise (Churchward-Venne et al., 2012). Decreases in peripheral macrovascular blood flow have been identified ≤ 60 min following immersion (Gregson et al., 2011; Mawhinney et al., 2013), and protein synthesis is highly correlated with muscle blood flow (Fujita et al., 2006). Therefore, a chronic accumulation of acute reductions in amino acid delivery as a result of decreased blood flow, may contribute toward an attenuated adaptation to resistance exercise.

Activity of the mTOR pathway (in particular p70S6K) and satellite cell accretion are known to play a large role in governing the hypertrophic and adaptive response to resistance exercise (Mayhew et al., 2009; Mitchell et al., 2013; Blaauw & Reggiani, 2014). Basic cellular and enzymatic reactions and mechanisms are known to be temperature sensitive, and operate on an "inverted-U" basis. Therefore, reductions in tissue and core temperature following immersion may acutely reduce the activity of such processes. Resistance exercise adaptation depends on an interaction between a number of cellular responses and pathways, with many being most active in the first 1–2 h following exercise. An accumulation of acute attenuation of such responses is likely to play a larger role in governing a decreased long-term adaptation.
Data from study 4 is in support of this theory. Responses that were attenuated after cold water immersion included the key mTOR pathway proteins, p70S6K and rpS6, as well as NCAM+ and PAX7+ satellite cells. Immersion did not affect another protein, 4E-BP1, which also regulates muscle protein synthesis. On the other hand, the phosphorylation of mitogen activated protein kinase ERK1 and ERK2 was increased 2 h following immersion, whilst no phosphorylation was evident following active recovery. Hypertrophic adaptation to resistance exercise correlates highly with p70S6K; therefore, the suppression of this kinase by cold water immersion could be detrimental to a hypertrophic response. The number of NCAM+ and PAX7+ satellite cells were decreased following immersion. As these cells have also been reported to play a key role in muscle hypertrophy, regular suppression satellite cell activity after resistance exercise could also reduce muscle adaptations.

In conclusion, acute muscle function following resistance exercise may be maintained following cold water immersion as a result of reduced tissue temperatures, and central and peripheral dynamics. In turn, these could reduce the perception of soreness, decrease oedema, enhance recovery of heart rate variability and the perception of recovery. Although these indices were accompanied by systemic and local oxygenation and metabolic responses, these were seemingly not detrimental to acute performance. However, important acute anabolic responses such as p70S6K activity and satellite cell accretion were attenuated by cold water immersion. These responses are vital contributors to muscular regeneration, and hypertrophic adaptation to resistance exercise. In the correct environment, such as between competitive heats or following a single competitive bout, when recovery maximisation is the goal, these responses may be overlooked. However, in an adaptive environment, such as within a training cycle, a regular repetition of these responses could bear significant weighing. Ultimately, a regular repetition of such responses could substantially diminish training adaptations.

5.2 Practical and methodological considerations

The data in this thesis cannot necessarily be extrapolated to elite or highly trained athletes. All participants included in the investigations contained within this thesis were from a homogenous exercise or training background, however these ranged from the recreationally active, to those recreationally experienced with resistance training. These participants were selected because it is often difficult to recruit elite or highly trained athletes to take part in invasive research, which requires a large time commitment. It is
possible that elite or highly trained athletes may have responded differently to cold water immersion compared with the physically active young men in this series of studies.

A second issue to consider is the use of active recovery as a control condition as opposed to a passive control when identifying the exact responses following cold water immersion. Active recovery (10 min at a “low, self selected intensity” of ~50 watts) was used in order to promote the ecological validity associated with the investigations, as it is unlikely that athletes would not perform any form of recovery following resistance exercise. It is questionable whether such a low intensity is of a sufficient intensity to be deemed true active recovery, however some of the differences observed between cold water immersion and active recovery conditions could be attributed to the effects of this level of active recovery per se. Active recovery may not be a popular recovery therapy implemented following resistance or combined exercise, compared with other therapies such as stretching or compression garments (Bahnert et al. 2013) or nutritional supplementation to enhance post-exercise protein synthesis (Koopman et al. 2007). Regardless of the uncustomary nature of using active recovery following resistance exercise, positive effects upon long-term adaptation to resistance training have been identified when utilising this method.

A final consideration is with regards to the potential contribution of the placebo effect. Evidence from investigations such as Bahnert et al. (2013) and Roberts et al. (2014) support the effectiveness for cold water immersion in increasing perceptions of recovery and reduced soreness after exercise. However, Broatch et al. (2014) recently reported that cold water immersion was no more effective in augmenting performance, inflammatory markers, and perceptions of recovery than a placebo condition. Therefore, it is unknown whether some of the results contained in this thesis could be attributed to a placebo effect.

5.3 Significance

To date, a limitation of the current knowledge base regarding the use of cold water immersion after resistance exercise has been the number of investigations on cold water immersion that involved severe muscle-damaging exercise. Although eccentric resistance exercise can be used to promote adaptation, it is unlikely to be the predominant training method for athletes. Therefore, traditional resistance exercise was used throughout this thesis, to allow more direct application to athletic training programs, and expand on the knowledge available in relation to this exercise type. The data contained within this thesis
lend support to using cold water immersion in a targeted and periodised manner, based on the nature of the preceding exercise bout. It may be appropriate to use cold water immersion between multiple training sessions in one day and/or competitive events in close succession, when rapid recovery is required to minimise fatigue and maintain performance. However, cold water immersion may be less appropriate following exercise bouts that are designed to promote muscular adaptation. Another limitation of the current knowledge base surrounding the use of cold water immersion following resistance exercise is the paucity of information available on the mechanisms of effect. The data contained in this thesis provide novel insights into the anabolic cellular and molecular responses that are influenced by this recovery therapy. Furthermore, the data also expands on current knowledge regarding some more global physiological responses, such as cardiac output, along with other more local responses, such as intramuscular oxygenation. These cellular, molecular and physiological responses following cold water immersion also warrant consideration when designing recovery strategies that incorporate cold water immersion.

5.4 Future directions

There is still much to learn about the optimal conditions for using cold water immersion as a recovery strategy. There is currently little consensus regarding the optimal water temperature, duration and depth of immersion, timing of immersion post-exercise and the frequency of immersion. More research is required to examine these factors in a systematic manner. It is important that future research includes some robust, valid and reliable measurements of muscle function and physiological processes. When designing future research, it will also be important to consider the ultimate goal of using cold water immersion, or more specifically, what physiological processes are targeted, and why.

Cold water immersion and other cryotherapies have traditionally been used to minimise inflammation. Yet, very little research, at least in humans, has specifically examined this issue. It is also unknown whether acute and/or long-term cold water immersion following resistance exercise influence genetic modulators of adaptation such as RNA and miRNA levels, and even epigenetic modification of DNA. Lastly, considering the effects of cold water immersion on central and peripheral blood flow, future research is required into whether cold water immersion affects the delivery of amino acids to skeletal muscle, and if so, whether protein fractional synthetic rate is influenced.
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Appendix 1. Ethical approvals.

Study 1.

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THE UNIVERSITY OF QUEENSLAND
Institutional Approval Form For Experiments On Humans
Including Behavioural Research

Chief Investigator:  Mr Llion Arwyn Roberts
Project Title:  Time Course Of Performance Recovery Following Cold Water Immersion
Supervisor:  Prof Jeff Coombes
Co-Investigator(s):  None
Department(s):  Human Movement Studies
Project Number:  2012000662
Granting Agency/Degree: Queensland Academy of Sport, Centre of Excellence
Duration:  31st December 2013

Comments:

Name of responsible Committee:--
Medical Research Ethics Committee
This project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:--
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date:  10 July 2012  Signature:  

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Study 2.

THE UNIVERSITY OF QUEENSLAND
Institutional Approval Form For Experiments On Humans
Including Behavioural Research

Chief Investigator: Mr Lilian Arwyn Roberts
Project Title: Time Course Of Performance Recovery Following Cold Water Immersion
Supervisor: Prof Jeff Coombes
Co-Investigator(s): None
Department(s): Human Movement Studies
Project Number: 2012000662
Granting Agency/Degree: Queensland Academy of Sport, Centre of Excellence
Duration: 31st December 2013

Comments:

Name of responsible Committee:-
Medical Research Ethics Committee
This project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date: 10 July 2012
Signature:
Study 3.

The University of Queensland
Institutional Approval Form for Experiments on Humans Including Behavioural Research

<table>
<thead>
<tr>
<th>Chief Investigator:</th>
<th>Mr Lilian Arwyn Roberts</th>
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</thead>
<tbody>
<tr>
<td>Project Title:</td>
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</tr>
<tr>
<td>Supervisor:</td>
<td>Prof Jeff Coombes</td>
</tr>
<tr>
<td>Co-Investigator(s):</td>
<td>None</td>
</tr>
<tr>
<td>Department(s):</td>
<td>Human Movement Studies</td>
</tr>
<tr>
<td>Project Number:</td>
<td>2012000662</td>
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<tr>
<td>Granting Agency/Degree:</td>
<td>Queensland Academy of Sport, Centre of Excellence</td>
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<tr>
<td>Duration:</td>
<td>31st December 2013</td>
</tr>
</tbody>
</table>

Comments: 

Name of responsible Committee: Medical Research Ethics Committee

This project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative: Professor Bill Vicenzino

Chairperson

Medical Research Ethics Committee

Date: 10 July 2012
Signature: [Signature]
THE UNIVERSITY OF QUEENSLAND
Institutional Approval Form For Experiments On Humans
Including Behavioural Research

Chief Investigator: Mr Lilin Arwyn Roberts
Project Title: Time Course Of Performance Recovery Following Cold Water Immersion
Supervisor: Prof Jeff Coombes
Co-Investigator(s): None
Department(s): Human Movement Studies
Project Number: 2012000662
Granting Agency/Degree: Queensland Academy of Sport, Centre of Excellence
Duration: 31st December 2013

Comments:

Name of responsible Committee:--
Medical Research Ethics Committee
This project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:--
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date: 10 July 2012 Signature: [Signature]

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Appendix 2. Participant information and informed consent forms.

Study 1.

**RESEARCH PARTICIPATION INFORMATION SHEET**

**PROJECT TITLE:** *Time course of performance recovery following cold water immersion*

**Researchers:**
- Llion Arwyn Roberts (School of Human Movement Studies, UQ)
- Prof. Jeff Coombes (School of Human Movement Studies, UQ)
- Dr. Jonathan Peake (School of biomedical sciences, QUT)

**Location:**
School of human movement studies
Blair Drive
St. Lucia
Brisbane
4072

**Contact info:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Tel no.</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llion Roberts</td>
<td>0733656983 / 0416686660</td>
<td><a href="mailto:l.roberts2@uq.edu.au">l.roberts2@uq.edu.au</a></td>
</tr>
<tr>
<td>Prof. Jeff Coombes</td>
<td>0733656767</td>
<td><a href="mailto:jcoombes@uq.edu.au">jcoombes@uq.edu.au</a></td>
</tr>
<tr>
<td>Dr. Jonathan Peake</td>
<td>0733794181</td>
<td><a href="mailto:Jonathan.peake@qut.edu.au">Jonathan.peake@qut.edu.au</a></td>
</tr>
</tbody>
</table>

**What is the aim of the study?**

Cold water immersion is a popular recovery strategy following exercise. Despite this popularity, it remains unclear how it affects recovery of muscle function after exercise. The aim of this study is to investigate the effects of cold water immersion on short-term recovery following high-intensity resistance exercise.

**Do I have to take part?**

The decision of whether to take part in this investigation is entirely optional. If you do decide that you would like to participate, you are asked to carefully read the following information, and sign a consent form. Any decision that you might make to withdraw from the investigation at any time will...
not in any way affect your rights, future treatment or service you will receive from the research team. You are free to withdraw from the investigation without prejudice at any time.

What will happen to me if I take part?
The study will involve three initial visits (for familiarisation and baseline testing), and two experimental trials that will take place at the visits to the physiology laboratory at the School of Human Movement Studies, UQ.
Each experimental trial will be conducted on separate days, and each trial will require approximately 8 hours of your time. The two trials will involve the following activities:

Exercise with cold water immersion: A lower body resistance training session for ~ 1hr followed by 10 min of cold water immersion at 10°C. Periodic performance measures over the following 6 hours.

Exercise without cold water immersion: Lower body resistance training session for ~ 1hr followed by 10 min of low-intensity cycling. Periodic performance measures over the following 6 hours.

As part of the baseline testing, you will undergo a DXA scan to assess your levels of lean body mass and fat mass. During the scan you will lie on a specially designed table whilst a scanning arm moves above your body. The scan time for the scan is approximately 7 minutes, during which, a low-dosage x-ray will pass from underneath the table to the scanning arm above you.

Also as part of the baseline testing, you will be tested to measure your strength while performing the back squat, front squat, incline leg press and knee extensions. You will also be tested to measure your performance in weighted and unweighted squat jumps.

As the last part of the baseline testing, you will complete a training simulation exercise. This consists of six sequential sets of 10 squat repetitions, at a weight corresponding to 80% of your maximum. Repetitions will be performed using a standard squat rack, and the number of successful repetitions noted per set.

At least a week after the baseline measures have been collected, you will perform the first out of your two experimental trials

For the week before your first experimental trial you will be allowed to train as normal, however we will ask you to record a diary of any exercise you perform. For the 24 hours before your first trial, you will need to refrain from any stimulants such as coffee or alcohol, and refrain from consuming any other supplements (if you’re unsure what supplements you shouldn’t be taking please ask).
For this 24 hour period you will also be asked to keep a diary of any food and drink you consume. You will need to bring your training and food diaries with you on your first experimental trial.

We will ask you to consume water (~ 600 – 800 ml) over the 2 hours before each of your two experimental trials to ensure that you are not dehydrated.

On your arrival for both your experimental trials, you will initially provide us with a small (~ 50 ml) urine sample, and a 30 ml blood sample will be taken from a vein in your arm. We will analyse the urine to check your hydration levels. A trained phlebotomist will also insert a cannula into a thigh muscle so that we can record your muscle temperature.

You will then perform a resistance training session consisting of similar exercises to those that you undertook during the baseline strength tests. The resistance exercise will be tiring, but similar to a typical lower body resistance training session.

At the end of the training session, we will measure your maximal strength again, and record your muscle temperature. You will then either immerse most of your body (to the neckline) for 10 min in an inflatable bath of cold water set at 10°C, or you will ride at a low-intensity on a stationary bicycle for 10 min.

Following the cold water immersion or cycling, you will rest quietly for a period of 6 hours. During this period, you will be given some food and drink, and you will be able to read or watch DVDs. We will record your muscle temperature, collect more urine and blood samples and measure your strength recovery after 2 hours and 4 hours. After 6 hours, you will complete the training simulation exercise described above. We will then collect a final blood and urine sample before the end of the trial. You will then be free to go home. We will give you back a copy of your training record, and food diary and ask you to replicate them over the days leading up to your second visit.

**What are the risks/benefits of my involvement in the study?**

DXA uses very low energy x-rays to determine body composition and bone mass. The dose associated with DXA studies of the whole body is 2.6 µSv. In comparison, individuals receive approximately 7 µSv from daily natural exposure, 80 µSv for a return trans-Pacific flight, 100 µSv for a chest x-ray, and 2000 µSv for a lumbar spine x-ray. Therefore, although radiation is used in the scan, the amount of radiation is very small and the corresponding risk from participating in this study is extremely low.
The resistance exercise components may cause you and your muscles to feel tired. However, this feeling should not be much different to how you feel following any regular resistance training session.

The cold water will reduce your body temperature, and may feel a little unusual when you first enter the water. This feeling should settle down fairly quickly, however, as your body gets used to the temperature. As the immersion time is short, you should not feel too much discomfort, and immediately after the immersion you will be allowed to towel dry in order to remove any excess water and warm up once more.

All blood collection and muscle temperature procedures will be performed by a trained phlebotomist, to minimize the chances of infection or any other adverse effects.

You will benefit from this research by firstly receiving a very accurate and in depth assessment of your body composition through the DXA scan. The research will also teach you about training habits targeting the lower body that may help you in your future training. The results of the investigation will also inform you about when it would be best for you to perform resistance exercise if you were to perform cold water immersion as a part of your recovery strategy in the future.

**Will my details be kept confidential?**
Your identity and confidentiality will be kept intact at all times for the duration of the study. You have the right to request information regarding any of the data collected regarding yourself at any time.

**Your rights**
This study has been cleared in accordance with the ethical review guidelines and processes of the University of Queensland. These guidelines are endorsed by the University’s principal human ethics committee, the Human Experimentation Ethical Review Committee, and registered with the Australian Health Ethics Committee as complying with the National Statement. You are free to discuss your participation in this study with project staff (contactable on 0733656877; Llion Roberts). If you would like to speak to an officer of the University not involved in the study, you may contact the School of Human Movement Studies Ethics Officer on 3365 6380 (Dr Tim Carroll).
RESEARCH PARTICIPATION INFORMATION SHEET

PROJECT TITLE: *Time course of performance recovery following cold water immersion*

Researchers: Llion Arwyn Roberts (School of Human Movement Studies, UQ)
Prof. Jeff Coombes (School of Human Movement Studies, UQ)
Dr. Jonathan Peake (School of biomedical sciences, QUT)

This study has been cleared in accordance with the ethical review guidelines and processes of the University of Queensland. These guidelines are endorsed by the University's principal human ethics committee, the Human Experimentation Ethical Review Committee, and registered with the Australian Health Ethics Committee as complying with the National Statement. You are free to discuss your participation in this study with project staff (contactable on 0733656877; Llion Roberts). If you would like to speak to an officer of the University not involved in the study, you may contact the School of Human Movement Studies Ethics Officer on 3365 6380 (Dr Tim Carroll).

1. I, the undersigned……………………………………………… hereby acknowledge that I have read the information document, and that the specific sections of the document that are relevant to the present experiment have been drawn to my attention. I have been provided with a description of the experiment, including the purposes, methods, demands, and possible risks and inconveniences involved.

2. I am aware that I may withdraw from this research project at any time without penalty (even after I have signed this statement of participation), and that I am entitled to a thorough explanation of any procedure employed in the study. I understand that any information I provide will be treated confidentially, and that it I will not obtain any direct benefits from my participation other than what has been outlined in the participant information sheet.

3. I hereby consent to being a research participant in this study.

(Signed)…………………………………………………………… Date: ……………………

(Witnessed by)………………………………………………… Date: ……………………
RESEARCH PARTICIPATION INFORMATION SHEET

Researchers:  
Llion Arwyn Roberts (School of Human Movement Studies, UQ)  
Prof. Jeff Coombes (School of Human Movement Studies, UQ)  
Dr. Jonathan Peake (School of Biomedical sciences, QUT)  
Dr. Mark Muthalib (Movement to Health Laboratory, University of Montpellier)  
Prof. Ken Nosaka (School of Health and Exercise Science, ECU)  

Location:  
School of human movement studies  
Blair Drive  
St. Lucia  
Brisbane  
4072  

Contact info:  
Tel no.  
Llion Roberts 0733656983 / 0416686660  
Prof. Jeff Coombes 0733656767  
Dr. Jonathan Peake 0731380542  
Email  
l.roberts2@uq.edu.au  
jcoombes@uq.edu.au  
jonathan.peake@qut.edu.au  

What is the aim of the study? 
Cold water immersion is a popular recovery strategy following exercise. Despite this popularity, it remains unclear how it affects the body, particularly cardiac output and skeletal muscle oxygen use. The aim of this study is to investigate how cold water immersion affects these variables after exercise.  

Do I have to take part? 
The decision of whether to take part in this investigation is entirely optional. If you do decide that you would like to participate, you are asked to carefully read the following information, and sign a consent form. Any decision that you might make to withdraw from the investigation at any time will not in any way affect your rights, future treatment or service you will receive from the research team. You are free to withdraw from the investigation without prejudice at any time.
What will happen to me if I take part?
The study will involve an initial visit for familiarisation, and the collection of baseline performance data. After this visit, a further two visits will be required, one for each experimental condition.

All trials will take place at the visits to the physiology laboratory at the School of Human Movement Studies, UQ. The visits for the experimental trials will take place on separate days, separated by at least 7 days. These two trials will involve the following activities:

Exercise with cold water immersion: A fatiguing knee extensor exercise bout targeting the quadriceps of your dominant leg followed by 10 min of cold water immersion of your lower body at 10°C. Periodic performance measures over the following 70 min.

Exercise without cold water immersion: A fatiguing knee extensor exercise bout targeting the quadriceps of your dominant leg followed by 10 min of stationary cycling at a low, self-selected intensity. Periodic performance measures over the following 70 min.

During the familiarisation/baseline visit, you will be familiarised with the equipment used during the trials. Two types of strength from your dominant leg will also be measured – your maximal static strength pushing against an immovable ‘arm’, and your strength during a repeated task pushing against an arm that will move at a constant velocity.

For the week before your first experimental trial you will be allowed to train as normal, however we will ask you to record a diary of any exercise you perform. For the 24 hours before your first trial, you will need to refrain from any stimulants such as coffee or alcohol, and refrain from consuming any other supplements (if you’re unsure what supplements you shouldn’t be taking please ask). For this 24 hour period, you will also be asked to keep a diary of any food and drink you consume. You will need to bring your training and food diaries with you on your first experimental trial.

We will ask you to consume water (~ 600 – 800 ml) over the 2 hours before each of your two experimental trials to ensure that you are not dehydrated.

On your arrival for both your experimental trials, a trained phlebotomist will insert a needle in to the thigh of your dominant leg so that we can record your muscle temperature, a fine-wire probe will then be fed through the needle, in to the muscle, before the needle is then removed, leaving the probe behind for the trial. A skin temperature probe will also be attached to the skin near where the needle was inserted / removed. Finally, to measure tissue oxygenation, a non-invasive method known as near infra-red spectroscopy will be used. This method involves placing a probe on the surface of the leg to measure the level of oxygen saturation in the muscle below the skin.
A total of 5 electrodes will also be placed on your upper body to measure cardiac output.

Maximal static strength will then be measured once more, before you perform a 25 min period of repetitive knee extension exercise with your dominant leg. This exercise will be fatiguing for the quadriceps, but is designed to increase heart rate, as well as blood flow to the quadriceps.

After the end of the exercise period, you will then either immerse yourself up to the waist for 10 min in an inflatable bath of cold water set at 10°C, or you will ride at a low, self-selected intensity on a stationary bicycle for 10 min.

Following the cold water immersion or cycling, you will rest for an hour. At 30 and 60 min in to the rest period, static strength will again be measured. At the end of the recovery period (~70 min), you will perform the same repetitive strength task as you completed during your baseline / familiarisation visit.

**What are the risks/benefits of my involvement in the study?**

The knee extension bout and repetitive strength task will cause the quadriceps muscle of your dominant leg to feel tired. However, this feeling should not be much different to how you feel following any regular resistance training session.

The cold water will reduce your body and muscle temperature, and may feel a little unusual when you first enter the water. This feeling should settle down fairly quickly as your body gets used to the temperature. As the immersion time is short, you should not feel too much discomfort, and immediately after the immersion you will be allowed to towel dry in order to remove any excess water and warm up once more.

The muscle temperature procedure will be performed by a trained phlebotomist, to minimize the chances of infection or any other adverse effects.

You will benefit from this research by helping the research team to perform this research. You will also gain a novel insight in to how your cardiac system responds to exercise and cold water immersion.

During the project, approximately 5 hours of your time will be required. Due to this time commitment, you will be reimbursed with $100 for your time, and other costs associated with participating in the study.
Will my details be kept confidential?
Your identity and confidentiality will be kept intact at all times for the duration of the study. You have the right to request information regarding any of the data collected regarding yourself at any time.

Your rights
This study has been cleared in accordance with the ethical review guidelines and processes of the University of Queensland. These guidelines are endorsed by the University's principal human ethics committee, the Human Experimentation Ethical Review Committee, and registered with the Australian Health Ethics Committee as complying with the National Statement. You are free to discuss your participation in this study with project staff (contactable on 0733656877; Llion Roberts). If you would like to speak to an officer of the University not involved in the study, you may contact the School of Human Movement Studies Ethics Officer on 3365 6380 (Dr Tim Carroll).
PROJECT TITLE: Cardiac output and localised muscle oxygenation responses to exercise and cold water immersion

Researchers: Llion Arwyn Roberts (School of Human Movement Studies, UQ)
Prof. Jeff Coombes (School of Human Movement Studies, UQ)
Dr. Jonathan Peake (School of Biomedical sciences, QUT)
Dr. Mark Muthalib (Movement to Health Laboratory, University of Montpellier)
Prof. Ken Nosaka (School of Health and Exercise Science, ECU)

This study has been cleared in accordance with the ethical review guidelines and processes of the University of Queensland. These guidelines are endorsed by the University's principal human ethics committee, the Human Experimentation Ethical Review Committee, and registered with the Australian Health Ethics Committee as complying with the National Statement. You are free to discuss your participation in this study with project staff (contactable on 0733656877; Llion Roberts). If you would like to speak to an officer of the University not involved in the study, you may contact the School of Human Movement Studies Ethics Officer on 3365 6380 (Dr Tim Carroll).

1. I, the undersigned…………………………………… hereby acknowledge that I have read the information document, and that the specific sections of the document that are relevant to the present experiment have been drawn to my attention. I have been provided with a description of the experiment, including the purposes, methods, demands, and possible risks and inconveniences involved.

2. I am aware that I may withdraw from this research project at any time without penalty (even after I have signed this statement of participation), and that I am entitled to a thorough explanation of any procedure employed in the study. I understand that any information I provide will be treated confidentially, and that it I will not obtain any direct benefits from my participation other than what has been outlined in the participant information sheet.

3. I hereby consent to being a research participant in this study.

(Signed) .......................................................... ........................................
(Witnessed by) .......................................................... ..............................
RESEARCH PARTICIPATION INFORMATION SHEET

PROJECT TITLE: Short and long term effects of resistance exercise and cold water immersion

Researchers: Llion Arwyn Roberts (School of Human Movement Studies, UQ) Prof. Truls Raastad (Norwegian School of Sport Science, Oslo, Norway) Prof. David Cameron-Smith (University of Auckland, Auckland) Dr. Glen Lichtwark (School of Human Movement Studies, UQ) Dr. Jonathan Peake (School of Biomedical sciences, QUT) Prof. Jeff Coombes (School of Human Movement Studies, UQ)

Location:
School of human movement studies
Blair Drive
St. Lucia
Brisbane
4072

Contact info: Tel no. Email
Llion Roberts 0733656983 / 0416686660 l.roberts2@uq.edu.au

What is the aim of the study?
Cold water immersion is a popular recovery strategy following exercise. Despite this popularity, it remains unclear how it affects long-term physiological and performance adaptation. The aim of this study is to investigate the short- and long-term effects of resistance exercise performed with (or without) cold water immersion.

Do I have to take part?
The decision of whether to take part in this investigation is entirely optional. If you do decide that you would like to participate, you are asked to carefully read the following information, and sign a consent form. Any decision that you might make to withdraw from the investigation at any time will not in any way affect your rights, future treatment or service you will receive from the research team. You are free to withdraw from the investigation without prejudice at any time.
What will happen to me if I take part?
The study will involve two parts, part (A) and part (B). You would be required for both parts.
Part A will involve a 12 week period of supervised resistance training within the strength and conditioning laboratory at the School of Human Movement Studies, while part B will involve the completion of a single further training session within the same laboratory. A 7-14 day period will exist between both parts.

Performance will be measured and muscle samples will be taken during the week before, and the week after the 12 weeks of training. Additional blood and muscle samples will be taken 4, 24 and 48 hours after the single training session of part B.

Based on your height, weight and lean mass, you will be allocated in to a cold water immersion or active recovery group. Consequently, you will perform 10 min of either cold water immersion at 10°C, or stationary low intensity cycling after each training session you complete.

Before, and after the 12 week training period is undertaken, the following measurements will be undertaken over 7 days:

Day 1, at the School of Human Movement Studies
   A DXA scan that will very accurately assess your whole body composition
   A measurement of the maximal static and dynamic strength of your right leg
   A measurement of the repetition maximum strength of your legs during lower body exercises

Day 2, at the Wesley Hospital, Brisbane
   An MRI scan that will accurately assess the composition of your dominant leg

Day 3, at the school of human movement studies
   A muscle biopsy from the thigh of your dominant leg

During the 12 weeks of training, you will complete a maximum of 36 training sessions, each separated by at least 48 hrs. In fortnightly blocks (after weeks 2, 4, 6, 8 and 10) we will measure your repetition maximum strength once more within a training session, in order to set your training intensities for the next two-week block.

Other than the pre training measures, you will refrain from any formal training for two weeks before the beginning of the training period.
After each training session you will be provided with a protein/carbohydrate recovery shake.

We will also advise you as to the amounts of protein, carbohydrate and fat that we would like you to try and consume daily, in order to allow you to get the most out of the training period, and for standardization.

Periodically over the training period, we’ll ask you to complete a dietary log so that we’re aware of the typical daily amounts of protein, carbohydrate and protein that you’re consuming.

We do not want you to stop exercising outside of the training, but we would like you to keep it to a minimum. We would like you to complete a basic weekly training log that details any formal training you do complete.

Between the end of the training period and the completion of part B of the study, we would like you not to complete any formal training other than the performance measures that you complete.

Part B will involve you completing a single, final training session. This session will be at a higher intensity than the training sessions during the previous 12 weeks.

Before the training session we’ll take a blood sample from a vein in the crease of one of your elbows. After you’ve completed the training session you will again be provided with a protein/carbohydrate shake. At 4, 24 and 48 hours after the session, we will take further blood samples, and we will also take a muscle biopsy from your thigh at the same time.

Over these 48 hours after the training session, if possible we would like you to refrain from any exercise. We would also like you to meet the daily suggested amounts of protein, carbohydrate and fat over this period. You will need to complete a dietary log over these 48 hours.

**What are the risks/benefits of my involvement in the study?**

The resistance exercise components may cause you and your muscles to feel tired. This feeling, however, should not be much different to how you feel following any regular resistance training session.

The cold water will reduce your body temperature, and may feel a little strange when you first enter the water, along with feelings of numbness in your feet. This feeling should settle down fairly quickly, however, as your body gets used to the temperature. As the immersion time is short, you should not feel too much discomfort, and immediately after the immersion you will be allowed to towel dry in order to remove any excess water and warm up once more.
All blood and muscle biopsy collections will be undertaken by an experienced and trained individual. This will minimise the chances of infection or any other adverse effects happening.

Local anaesthetic will be applied to your thigh before each biopsy to minimise any pain during the procedure. You may feel some slight discomfort during the procedure, but this feeling will subside quickly. A compression bandage will be applied after the biopsy is collected to enhance recovery. The biopsy collection will not stop you from performing any activity after collection, whilst any discomfort felt after the procedure will subside within ~ 24 hrs.

Each DXA will take approximately 7 min to complete, and will involve a small amount of radiation. The amount of radiation you would be exposed to (2.6 µSv) is much less radiation than individuals are exposed to daily from natural exposure (7 µSv), a chest x-ray (100 µSv) or a lumbar spine x-ray (2000 µSv). During the MRI procedure, you may feel slightly claustrophobic whilst you are in the magnet. However, this procedure will only take 15 min, and you will be able to press a panic alarm to end the procedure if you feel uncomfortable. You will be able to listen to music and close your eyes during the procedure to minimise any discomfort.

You will benefit from this research by firstly receiving very accurate and detailed assessments of your overall and localised body composition through the DXA and MRI scans.

You will also receive a 12 week period of tailored and supervised resistance training. This training is guaranteed to increase your muscular strength, muscular mass, and enhance your body composition. The research will also teach you about training habits that may help you in your future training. The results of the investigation will also inform you about how cold water immersion will affect your body and performance if you were to include it in to your regular training regime in the future.

Due to the time commitment involved in participating, you will also be paid $100 to compensate you for any loss of income or travel costs associated with participating in the study.

Will my details be kept confidential?
Your identity and confidentiality will be kept intact at all times for the duration of the study. You have the right to request information regarding any of the data collected regarding yourself at any time.

Your rights
This study has been cleared in accordance with the ethical review guidelines and processes of the University of Queensland. These guidelines are endorsed by the University’s principal human
ethics committee, the Human Experimentation Ethical Review Committee, and registered with the Australian Health Ethics Committee as complying with the National Statement. You are free to discuss your participation in this study with project staff (contactable on 0733656877; Llion Roberts). If you would like to speak to an officer of the University not involved in the study, you may contact the School of Human Movement Studies Ethics Officer on 3365 6380 (Dr Judy Bauer).
RESEARCH PARTICIPATION CONSENT FORM

PROJECT TITLE: Short and long term effects of resistance exercise and cold water immersion

Researchers:
Llion Arwyn Roberts (School of Human Movement Studies, UQ)
Prof. Truls Raastad (Norwegian School of Sport Science, Oslo, Norway)
Prof. David Cameron-Smith (University of Auckland, Auckland)
Dr. Glen Lichtwark (School of Human Movement Studies, UQ)
Dr. Jonathan Peake (School of Biomedical sciences, QUT)
Prof. Jeff Coombes (School of Human Movement Studies, UQ)

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1. I, the undersigned……………………………… hereby acknowledge that I have read the information document, and that the specific sections of the document that are relevant to the present experiment have been drawn to my attention. I have been provided with a description of the experiment, including the purposes, methods, demands, and possible risks and inconveniences involved.

2. I am aware that I may withdraw from this research project at any time without penalty (even after I have signed this statement of participation), and that I am entitled to a thorough explanation of any procedure employed in the study. I understand that any information I provide will be treated confidentially, and that it I will not obtain any direct benefits from my participation other than what has been outlined in the participant information sheet.

3. I hereby consent to being a research participant in this study.

(Signed) ………………………………………… Date: …………………

(Witnessed by) ………………………………………… Date: …………………
RESEARCH PARTICIPATION INFORMATION SHEET

PROJECT TITLE: The effect of cold water immersion on post-exercise inflammation and protein synthesis

Researchers:
Llion Arwyn Roberts (School of Human Movement Studies, UQ)
Prof. Truls Raastad (Norwegian School of Sport Science, Oslo, Norway)
Prof. David Cameron-Smith (University of Auckland, Auckland)
Dr. Jonathan Peake (School of Biomedical sciences, QUT)
Prof. Jeff Coombes (School of Human Movement Studies, UQ)

Location:
School of human movement studies
Blair Drive
St. Lucia
Brisbane
4072

Contact info:
Tel no. Email
Llion Roberts 0733656983 / 0416686660 l.roberts2@uq.edu.au

What is the aim of the study?
Cold water immersion is a popular recovery strategy following exercise. Despite this popularity, it remains unknown how it affects the short-term response to resistance exercise. The aim of this study is to investigate how the muscle responds to resistance exercise performed with (or without) cold water immersion.

Do I have to take part?
The decision of whether to take part in this investigation is entirely optional. If you do decide that you would like to participate, you are asked to carefully read the following information, and sign a consent form. Any decision that you might make to withdraw from the investigation at any time will not in any way affect your rights, future treatment or service you will receive from the research team. You are free to withdraw from the investigation without prejudice at any time.
What will happen to me if I take part?
The study will involve the completion of two separate ~ 30 min one-legged resistance training sessions, during two separate visits to the human performance laboratory at The School of Human Movement Studies. At least seven days will exist between both visits. Prior to your first visit, a measure of strength from each leg will be made, along with an assessment of your body composition.

Each visit will involve you performing a training session on one leg. If you exercise your left leg during your first visit, your right leg will be exercised during your second visit. After both training sessions you will be randomly assigned to undertake 10 min of either seated cold water immersion at 10°C, or stationary cycling at an intensity of your choice. If you perform the water immersion on your first visit, you will perform the stationary cycling during your second visit, or vice versa.

Before, after, and 0.5, 1, 2, 4, 24 and 48 hrs after each training session we’ll take a blood sample from a vein in the crease of one of your elbows. Before, and within 2, 12 and 48 hrs of the exercise, we will also take a muscle biopsy from the thigh of the leg which performed the training session. At the same time, we’ll measure the strength in your legs once more. Over the first 6 hours of recovery during each trial, you will be needed to remain at the laboratory to be monitored, and provided with water, protein and carbohydrate to consume.

We would like you to refrain from any lower body resistance exercise between both exercise trials, and we would like you to complete an exercise log detailing any formal exercise that you complete over the same time.

What are the risks/benefits of my involvement in the study?
The resistance exercise component may cause you and your muscles to feel tired. This feeling, however, should not be much different to how you feel following any regular resistance training session.

The cold water will reduce your body temperature, and may feel a little strange when you first enter the water, along with feelings of numbness in your feet. This feeling should settle down fairly quickly, however, as your body gets used to the temperature. As the immersion time is short, you should not feel too much discomfort, and immediately after the immersion you will be allowed to towel dry in order to remove any excess water and warm up once more.

All blood and muscle biopsy samples will be undertaken by an experienced, and trained individual to minimise the chances of infection or any other adverse effects happening.
Local anaesthetic will be applied to your thigh before each biopsy to minimise any pain during the procedure. You may feel some slight discomfort during the procedure, but this feeling will subside quickly. A compression bandage will be applied after the biopsy is collected to enhance recovery. The biopsy collection will not stop you from performing any activity after collection, whilst any discomfort felt after the procedure will subside within ~ 24 hrs.

Each DXA will take approximately 7 min to complete, and will involve a small amount of radiation. The amount of radiation you would be exposed to (2.6 μSv) is much less radiation than individuals are exposed to daily from natural exposure (7 μSv), a chest x-ray (100 μSv) or a lumbar spine x-ray (2000 μSv).

You will benefit from this research by firstly receiving very accurate and detailed assessments of your overall and localised body composition through the DXA scans.

Due to the time commitment involved in participating, you will also be paid $250 to compensate you for any loss of income or travel costs associated with participating in the study.

Will my details be kept confidential?
Your identity and confidentiality will be kept intact at all times for the duration of the study. You have the right to request information regarding any of the data collected regarding yourself at any time.

Your rights
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RESEARCH PARTICIPATION CONSENT FORM

PROJECT TITLE: The effect of cold water immersion on post-exercise inflammation and protein synthesis

Researchers: Llion Arwyn Roberts (School of Human Movement Studies, UQ)
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(Signed) ................................................................. Date: ...............................

(Witnessed by) .......................................................... Date: ...............................

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