Turning Up the Heat: Can Post-Exercise Hot Water Immersion Be Used to Manipulate Acute Physiological Responses & Chronic Adaptation Following Resistance Training?

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Abstract

Resistance training is a modality of exercise that is a staple part of strength and conditioning programmes as it offers benefits to competitive performance. Despite the positive adaptations which occur through performing regular training sessions over time, a single bout of resistance exercise results in a series of acute physiological responses. These may negatively impact performers in the hours and days post-exercise, although several questions exist with regards to appropriately characterising the magnitude and time course of this response which relate to the sensitivity of the measures which are used to do so. The context-dependent dichotomy between recovery and adaptation has fuelled much discussion in the scientific literature and has recently been articulated within the concept of hormesis, with post-exercise strategies aiming to optimise the exercise stimulus. The complex interplay between acute physiological responses and recovery/adaptation requires further investigation as recovery remains one of the least understood aspects of the exercise-adaptation cycle. Hot water immersion (HWI) is a form of heat therapy which is anecdotally reported to be used by athletes, whilst the modern advent of Jacuzzis and immersion pools in an increasing number of leisure facilities make it an easily accessible strategy. HWI may influence acute physiological responses within the recovery/adaptation paradigm but has received limited attention, while no research has investigated the chronic use of HWI alongside a resistance training programme. Therefore, the aim of this course of investigations was to elucidate the effects of HWI on acute physiological responses as well as recovery/adaptation to resistance exercise in a trained cohort. This research initially critically evaluated the literature investigating the use of HWI to identify several gaps worthy of further investigation. Subsequently, three experimental chapters were designed and conducted to assess the impact of HWI to manipulate acute physiological responses following resistance exercise and the influence on recovery/adaptation.

Study 1: The aim of this investigation was to assess the usefulness of a variety of measures that are used to detail acute physiological responses following resistance exercise. The study utilised a crossover design, assessed measures through a relevant timescale (i.e. 2 h – 96 h post-exercise), recruited trained participants and employed a real-world exercise modality to enhance the ecological validity of the findings. The results suggest that several measures were able to demonstrate clear effects following resistance exercise. Additionally, the results provided a profile relating to the magnitude of change and time course for these measures with optimal sampling points identified which informed the acute physiological response measures used in subsequent chapters.

Study 2: The aim of this study was to investigate the effect of HWI on acute physiological responses and recovery following resistance exercise. The main findings demonstrated that HWI is a viable means of heat therapy that can support a greater intramuscular temperature following resistance exercise. The elevated intramuscular temperature may have manipulated inflammatory processes. Although changes in other acute physiological response markers were independent of changes in intramuscular temperature associated with HWI. These results represent the first investigation into the acute physiological responses of a 'real-world' HWI protocol following resistance exercise, alongside the use of a trained cohort, applied exercise session and utilising good nutritional practice.

Study 3: This chapter aimed to investigate the effect of HWI on acute physiological responses and training adaptation following a 10-week resistance training programme. The main findings demonstrated that HWI (i) augmented long-term gains in strength, (ii) had no effect on the post-training increase in lower body lean mass (iii) elicited an accelerated recovery of muscle function and soreness in the acute post-exercise period following training, and (iv) attenuated the increase of markers of inflammation and muscle cell disruption following training compared to passive recovery (PAS). Collectively, these findings suggest that at the end of a 10-week training programme, HWI manipulates acute physiological responses to hasten post-exercise recovery. This may have positively impacted an individual's ability to train in subsequent sessions, leading to an accumulated training stimulus that induced small but worthwhile improvements in strength.

This course of investigation has provided novel information as to how HWI manipulates acute physiological responses and the subsequent impact on recovery/adaptation following resistance exercise. In addition to identifying sensitive measures and recommended sampling points for acute physiological responses, this research provides the first evidence which suggests (i) a 'real world' HWI protocol can maintain an elevated intramuscular temperature and blood flow following resistance exercise, (ii) acute physiological responses can be manipulated by HWI to enhance recovery during a training programme, and (iii) the HWI-associated benefits to training enabled small but worthwhile enhancements in strength adaptations following a resistance training programme. This series of studies utilised a 'real-world' HWI protocol, alongside the use of a trained cohort, applied exercise session and good nutritional practice, enhancing the ecological validity of this thesis. Further work is warranted to optimise the HWI protocol and widen the scope of application to other cohorts and with different exercise modalities, as well as deepen mechanistic knowledge. However, the positive findings from this thesis provide physiologists with rationale for utilisation of HWI alongside resistance training in their applied practice.

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As Nelson Mandela said, “It always seems impossible until it’s done”. Completing a PhD has certainly seemed impossible at times and I have experienced the full extent of the ups and downs along the way. This process has pushed me further than I could have imagined, and it has shaped the person that I am today. Getting to this stage is my proudest achievement, however, it is with the support of several people that it has been made possible.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work.

Name:

Signature:

Date:

Publications

Peer reviewed publications arising from this course of investigation

Jackman, J. S., Bell, P. G., Gill, S., van Someren, K., Davison, G. W., and Cockburn, E. (2018) 'Assessing the usefulness of acute physiological responses following resistance exercise: sensitivity, magnitude of change and time course of measures' *Applied Physiology, Nutrition and Metabolism,* (in press).

Conference communications and published abstracts arising from this course of investigation

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List of Abbreviations

The following abbreviations have been defined in the text in the first instance:

μM	Micromolar units
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BFR	Blood-flow restriction
Ca^{2+}	Calcium
CHO	Carbohydrate
CK	Creatine kinase
CK-BB	Creatine kinase (brain isoform)
CK-MB	Creatine kinase (cardiac isoform)
CK-MM	Creatine kinase (skeletal muscle isoform)
CMJ	Countermovement jump
CMJ_{PF}	Countermovement jump peak force
COX	Cyclooxygenase
CRP	C-reactive protein
CV	Coefficient of variation
CWI	Cold water immersion
DALDA	Daily analysis of life demands for athletes
DJ	Drop jump
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DXA	Dual-energy x-ray absorptiometry
E-C	Excitation-contraction
ECM	Extracellular matrix
EDTA	Di-potassium ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EPR	Electron paramagnetic resonance
FOX1	Ferrous oxidation of xylenol orange 1
HHb	Deoxyhaemoglobin
hsCRP	High sensitivity c-reactive protein
HWI	Hot water immersion
IGF-1	Insulin-like growth factor
IL-10	Interleukin-10
IL-1ra	Interleukin-1 receptor agonist
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
IL-8	Interleukin-8
IMT	Intramuscular temperature
ISO	Isometric squat
LDH	Lactate dehydrogenase
LOOH	Lipid hydroperoxides
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinase
MMP-9	Matrix metalloproteinase-9
MS	Muscle soreness
mTOR	Mammalian target of rapamycin
MuRF-1	Muscle ring finger 1
MVC	Maximal voluntary contraction
MVIC	Maximal voluntary isometric contraction

N	Newtons
NSAID	Non-steroidal anti-inflammatory drugs
O_2Hb	Oxyhaemoglobin
PAS	Passive recovery
PC	Protein carbonyls
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PLA_2	Phospholipase
RBE	Repeated bout effect
RFD	Rate of force development
RM	Repetition maximum
ROM	Range of motion
ROS	Reactive oxygen species
SD	Standard deviation
sIL-6R	Soluble interleukin-6 receptor
SmO_2	Tissue saturation of oxygen
SSC	Stretch-shortening cycle
sTnI	Skeletal troponin I fast form
tHb	Total haemoglobin
TNF-α	Tumour necrosis factor alpha
VAS	Visual analogue scale
WASO	Wake after sleep onset

1 Introduction

1.1 Introduction

Resistance training is a modality of exercise that can elicit increases in muscle size (Fry, 2004; Schoenfeld, Ogborn, & Krieger, 2016) and strength (Kraemer et al., 2002; Peterson, Rhea, & Alvar, 2004). These physical traits are recognised as being fundamental to health, functional ability and quality of life (Kraemer et al., 2002). For athletic populations, benefits can be seen relating to a range of sporting events and are therefore a staple of strength and conditioning programmes (Haff & Triplett, 2015). It is generally understood that these adaptations come about through performing regular bouts of resistance exercise and over time increases in muscle size and strength combine to allow individuals to perform at a greater level than before. However, a single bout of resistance exercise results in a series of acute physiological responses which include reduced muscle function, muscle soreness, inflammation and oxidative stress which may negatively impact performers in the hours and days post-exercise (Howatson & Van Someren, 2008).

The acute physiological responses that typify the period following a bout of resistance exercise are not only specific to this modality but are also dictated by other factors such as age, sex and training status (Howatson & Van Someren, 2008). Although this process is generally accepted (Clarkson & Hubal, 2002), measures of the acute physiological response exhibit inter-individual and diurnal variation (Miles et al., 2008), with the reliability rarely assessed across a time period that is reflective of that in which the measure is to be used (Atkinson & Nevill, 1998). To be confident that changes in these measures are real and not just due to random error, an understanding of the measures usefulness which incorporates the magnitude of change post-exercise compared with the typical error (signal:noise) is important (Pyne, Trewin, & Hopkins, 2004). Therefore, several questions exist with regards to appropriately characterising the magnitude and time course of this response which relate to the sensitivity of the measures which are used to do so. However, without information as to the most sensitive measures to characterise acute physiological responses and an understanding of 'real' changes, it is difficult to ascertain if interventions are modulating these responses.

A range of strategies exist with the aim of attenuating these acute physiological responses and accelerating recovery from exercise. On the other hand, it has been identified that these acute physiological responses play a role in driving the adaptive response (Schoenfeld, 2012), to which recent reports have demonstrated recovery strategies may inhibit training adaptation (Michailidis et al., 2013; Roberts et al., 2015; Trappe et al., 2002). The context-dependent dichotomy between recovery and adaptation has fuelled much discussion in the scientific literature and has recently been articulated within the concept of hormesis (Radak, Chung, Koltai, Taylor, & Goto, 2008). Strategies may either dampen or enhance physiological responses within hormesis with the goal of optimising the exercise stimulus (Peake,

Markworth, et al., 2015). However, the complex interplay between acute physiological responses and recovery/adaptation requires further investigation as recovery remains one of the least understood aspects of the exercise-adaptation cycle (Peake & Gandevia, 2017). Applied physiologists are therefore challenged with presenting how strategies manipulate acute physiological responses when looking to optimise strategies to prioritise either recovery and/or adaptation to resistance exercise.

Hot water immersion (HWI) is a form of heat therapy which may influence acute physiological responses within the recovery/adaptation paradigm but has received limited attention. HWI is anecdotally reported to be used by athletes (Vaile, Halson, & Graham, 2010), whilst the modern advent of Jacuzzis and immersion pools in an increasing number of leisure facilities make it an easily accessible strategy. The primary effects of HWI are suggested to be related to an increase in intramuscular temperature which induces peripheral vasodilation and a subsequent increase in tissue blood flow (Wilcock, Cronin, & Hing, 2006). Such an increase in tissue blood flow may facilitate increases in metabolism, nutrient delivery and waste removal through an increased permeability of cellular, lymphatic and capillary vessels (Wilcock et al., 2006).

Despite these effects which would in theory aid exercise recovery, the HWI literature is equivocal in this domain. Some investigators have reported benefits (Vaile, Halson, Gill, & Dawson, 2008b; Viitasalo et al., 1995), whilst others have shown no effects (Kuligowski, Lephart, Giannantonio, & Blanc, 1998; Pournot et al., 2011; Vaile, Halson, Gill, & Dawson, 2008a), however differences in HWI protocols, timing of application and exercise modality make conclusions problematic. The primary effects of HWI related to intramuscular temperature and tissue blood flow as well as the influence on acute physiological responses also need to be addressed with ecologically valid protocols. On the other hand, several reports have shown heat therapy to upregulate key anabolic pathways and protein expression (Goto et al., 2003; Kakigi et al., 2011), which may be related to the associated increases in tissue blood flow (Fujita et al., 2007). To date, no research has investigated the chronic use of HWI alongside a resistance training programme. The findings from related fields suggest that HWI may be a viable adjunct to resistance exercise in augmenting adaptation. However further research is required to provide applied physiologists with information as to how HWI influences acute physiological responses and the subsequent impact on recovery and adaptation in real world scenarios. Consequently, the overarching aim of this thesis was to assess the efficacy of HWI as a strategy to influence acute physiological responses in a trained cohort following resistance exercise within the context of the recovery/adaptation paradigm. This course of research is divided into three experimental chapters and set out to achieve the following overarching aims:

1. Identify sensitive measures and recommended sampling points for acute physiological responses following resistance exercise.
2. Provide a profile to characterise the magnitude of change and time course of the acute physiological response following resistance exercise.
3. Characterise the hypothesised physiological mechanisms (intramuscular temperature and muscle blood flow) to a practical HWI protocol.
4. Assess the effect of HWI on acute physiological responses and recovery following resistance exercise.
5. Investigate the influence of regular HWI on training adaptation following 10 weeks of resistance training.
6. Examine the effect of HWI on acute physiological responses following a 10-week resistance training programme.

2 Literature Review

2.1 Introduction

This literature review discusses acute physiological responses to resistance exercise with specific regard to the mechanisms that cause them and the measures that are used to assess them. The review then critically evaluates the role of these acute physiological responses in recovery from, and adaptation to, resistance exercise. Before discussing the use of HWI; to manipulate acute physiological responses following resistance exercise, and to impact upon recovery and adaptation.

2.2 Mechanisms of Acute Physiological Responses

Resistance exercise is a well-established method of training that results in beneficial adaptations such as increased skeletal muscle size (Fry, 2004; Schoenfeld et al., 2016) and strength (Kraemer et al., 2002; Peterson et al., 2004). Despite these positive alterations following a period of training, it is recognised that individuals may suffer negative consequences in the days post-exercise because of a single resistance exercise session. Indications of this transient response include; reduced muscle function, increased muscle soreness, muscle swelling, a reduced range of motion and increased accumulation of intramuscular proteins as well as markers of inflammation and oxidative stress in the blood. A bi-phasic model of this response has been proposed which incorporates; a primary phase related to incidents taking place during the exercise bout, and a secondary phase describing the cascade of events initiated as a result of inflammatory processes (Howatson & Van Someren, 2008).

2.2.1 Primary Response

The primary stress has typically been further divided into two possible pathways; metabolic and mechanical (Armstrong, Warren, & Warren, 1991). A third arm incorporating exercise that cannot be solely classified as either metabolic or mechanical, for example high-intensity intermittent exercise seen in team sports, may be referred to as a 'mixed' stress. Resistance exercise is most reflective of the mechanical pathway which relates to direct consequences as a result of loading myofibres, void of a contribution from metabolic factors (Howatson & Van Someren, 2008). This review will therefore focus on primary stress because of the mechanical pathway (Figure 2.1).

Resistance training is characterised by exercises that involve both concentric and eccentric muscle actions, and while both aspects may contribute, the eccentric component is suggested to be primarily responsible for the mechanical muscle stress (Howatson & Van Someren, 2008). Eccentric actions occur when the muscle is lengthened whilst simultaneously producing tension (Armstrong et al., 1991) and are capable of producing greater torque than other types of contraction (Dudley, Tesch, Miller, & Buchanan, 1991). This is despite a

smaller metabolic requirement and neural recruitment per unit of torque (Beltman, Van Der Vliet, Sargeant, & De Haan, 2004; Enoka, 1996; Newham, McPhail, Mills, & Edwards, 1983), which may cause a greater mechanical stress per fibre.

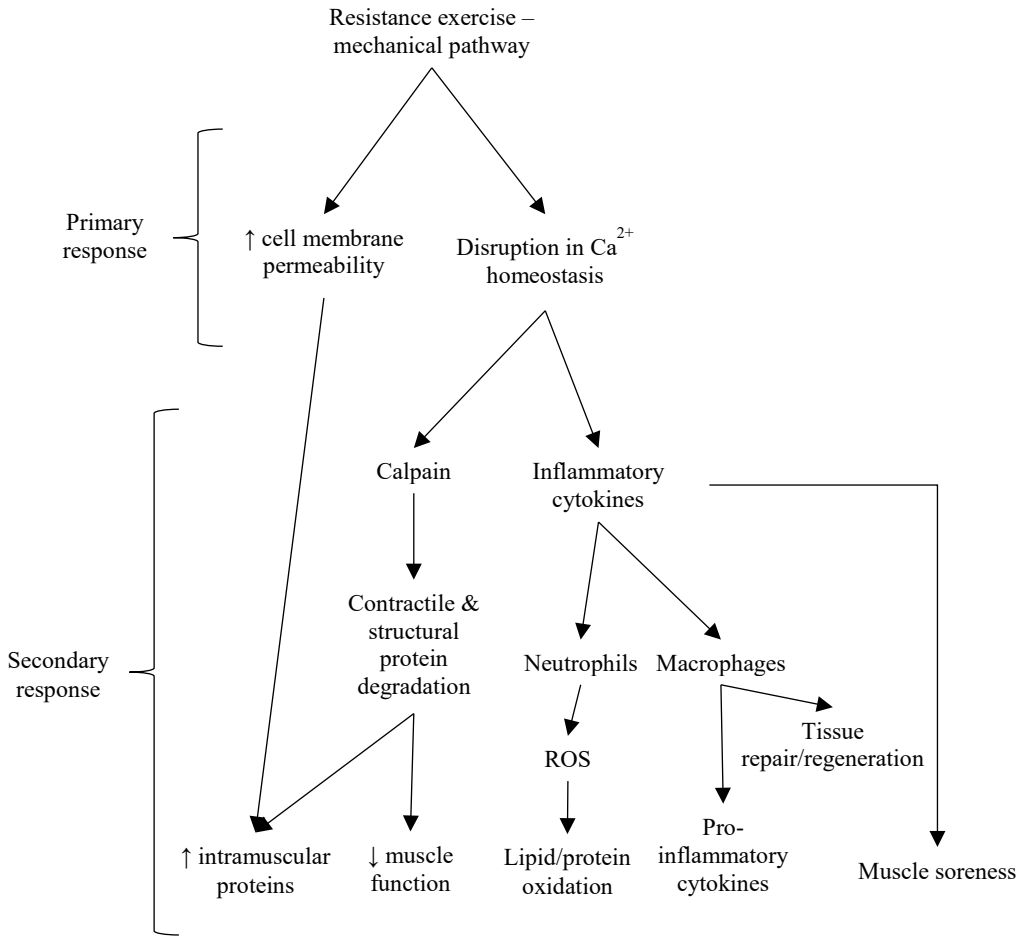

Figure 2.1 Overview of acute physiological responses.

The theoretical proposal for how eccentric actions translate into subsequent acute physiological responses has been described by the "popping sarcomere hypothesis" (Morgan, 1990). Variable lengthening of sarcomeres, consistent with eccentric actions, results in a reduced ability for cross-bridge formation with the weakest sarcomeres preferentially stretched (Morgan & Allen, 1999). Beyond optimum length, sarcomeres become progressively weaker as passive tension compensates for a reduction in active tension until filaments are stretched beyond the point of overlap, at which point "popping" occurs (Morgan & Proske, 2004). Resultant consequences include; Z-band streaming (Friden, Sjöström, & Ekblom, 1983), loss of calcium homeostasis (Yeung, Balnave, Ballard, Bourreau, & Allen, 2002), deformations to myofibrils and loss of membrane integrity (Morgan & Proske, 2004).

Failure of this structure manifests as a reduced ability of the muscle to generate force and together may be classified as the primary response (Howatson & Van Someren, 2008).

An alternative theory exists as to the initial event in which there is damage to the excitation-contraction (E-C) coupling system which may also contribute to impaired muscle function (Proske & Morgan, 2001). The level to which either of these processes represent the primary event remains controversial, with some suggesting that at least 75% of initial force losses are attributable to failure of the E-C coupling process (Warren, Ingalls, Lowe, & Armstrong, 2001). Support for this view is evidenced by augmented recovery of force when mouse fibres were potentiated with caffeine to recuperate E-C coupling processes (Balnave & Allen, 1995; Warren et al., 1993). The extent to which sarcomere disruption or E-C uncoupling contribute to the primary response is yet to be elucidated and an involvement of both theories remains possible.

2.2.2 Secondary Response

In relation to the direct consequences from the primary response, increased intracellular calcium (Ca^{2+}) is suggested to be the major candidate propagating a secondary response (Yeung et al., 2005). Extracellular Ca^{2+} exists in far greater concentrations than that of the cytosol (2-3 mmol/L vs. 0.1 μmol/L), resulting in a large influx into the cell following damage to the sarcolemma (Armstrong et al., 1991). Increased intracellular Ca^{2+} has been shown to reduce the force generating capacity of muscle (Balnave & Allen, 1995; Yeung et al., 2005), whilst also initiating a number of degradative pathways (Tee, Bosch, & Lambert, 2007).

Calpain and phospholipase A_2 (PLA_2) are Ca^{2+} activated enzymes with roles in proteolytic and phospholipolytic pathways, respectively, and contribute to the degradation of contractile and structural proteins (Tee et al., 2007). Plasma membrane proteolysis and myofibrillar protein degradation via calpain and PLA_2 activation has been shown to occur in skeletal muscle, which may explain the increased permeability of the sarcolemma following exercise (Belcastro, 1993; Jackson, Jones, & Edwards, 1984; Zaidi & Narahara, 1989). Support for the role of disturbed Ca^{2+} homeostasis in the secondary response is demonstrated by pharmacological blockage of Ca^{2+} channels which has been shown to reduce indications of muscle degeneration following eccentric exercise (Duarte, Soares, & Appell, 1992). It has been proposed that there is a relationship between calpain stimulated proteolysis and the accumulation and localisation of neutrophils following exercise which may be attracted through chemotactic signals (Raj, Booker, & Belcastro, 1998). Additionally, the PLA_2 liberation of arachidonic acid is a precursor to pro-inflammatory eicosanoids which are generated through the cyclooxygenase (COX) reaction (Balsinde, Winstead, & Dennis, 2002). Production of pro-inflammatory eicosanoids such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) play an additional

role in the process of inflammation and contribute to muscle protein synthesis and degradation, respectively (Trappe & Liu, 2013). PGE_2 has been shown to regulate interleukin-6 (IL-6) as well as the ubiquitin ligase muscle ring finger 1 (MuRF-1), both of which are implicated in the process of muscle atrophy (Trappe & Liu, 2013). Together calpain and PLA_2 initiate degenerative processes following an exercise bout which induce a further cascade of inflammatory events, particularly when the exercise stimulus is novel (Figure 2.1).

Following a loss of Ca^{2+} homeostasis, increased cell production of pro-inflammatory cytokines such as interleukin 1beta (IL-1β) and tumour necrosis factor alpha (TNF-α) may occur (Butterfield, Best, & Merrick, 2006). These cell signalling molecules have been shown to upregulate the expression of endothelial-leukocyte adhesion molecules within the endothelium of neighbouring blood vessels (Bevilacqua, Pober, Mendrick, Cotran, & Gimbrone, 1987; Pober, Bevilacqua, et al., 1986; Pober, Gimbrone, et al., 1986). Recent work has questioned the role of TNF-α in the post-exercise inflammatory response with research showing no response to eccentric actions (Malm et al., 2000; Steensberg et al., 2002), highlighting the importance of IL-1β in the early phase. At the site of endothelium activation, there is further release of IL-1β, as well as other pro-inflammatory cytokines IL-6 and interleukin-8 (IL-8) (Butterfield et al., 2006). IL-6 and IL-8 may act in a chemoattractant fashion thereby drawing neutrophils to the region (Detmers et al., 1991; Kaplanski et al., 1994; Liu & Spolarics, 2003). Whilst increased circulating levels of IL-6 are suggested to be the primary inducer of the anti-inflammatory cytokines interleukin-1 receptor agonist (IL-1ra) and interleukin-10 (IL-10) as well as hepatic acute-phase proteins (e.g. c-reactive protein) (Pedersen, Steensberg, & Schjerling, 2001; Steensberg, 2003).

Neutrophils are the first subpopulation of white blood cells to relocate to the site of tissue stress and accumulate rapidly following the initial insult (Cannon & Pierre, 1998). Evidence suggests neutrophils are phagocytic cells, initially recruited to breakdown damaged myofibres and clear cellular debris as well as releasing proteases which degrade the extracellular matrix (ECM) (Kuipers, Drukker, Frederik, Geurten, & Kranenburg, 1983). Support for the relationship between IL-1β and neutrophils is provided by research showing that following eccentric exercise-induced intracellular Z-band damage, the accumulation of IL-1β induced neutrophil chemotaxis and adhesion occurs (Fielding et al., 1993). The phagocytic function of neutrophils arises from their ability to release excessive levels of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals, which are non-specifically toxic and so may additionally harm nearby uninjured cells, exacerbating the primary response (Scott, Khan, Roberts, Cook, & Duronio, 2004). Inhibition of neutrophil actions has been shown to accelerate mouse wound repair (Dovi, He, & DiPietro, 2003) and reduce evidence of post-exercise muscle fibre injury (Brickson et al., 2003). In contrast, evidence for the role of

neutrophils aggravating the primary response is less clear in exercise models of eccentric actions, which have been unable to demonstrate a relationship between neutrophils and the secondary response (Armstrong, Ogilvie, & Schwane, 1983; Lapointe, Frenette, & Côté, 2002). Despite the controversial role of neutrophils in worsening the stress imposed on skeletal muscle, their initial relocation to the region pre-dates infiltration of the second major immune cells, macrophages (Butterfield et al., 2006).

Monocytes migrate to the site of stress and upon entering the tissue, mature and differentiate into macrophages (Cannon & Pierre, 1998). Macrophages are predominant during the latter stages of the inflammatory response and are activated by pro-inflammatory cytokines, including IL-1β (Hirani et al., 2001). It is now commonly accepted that macrophages exist in two cell populations; (i) $ED1^+$ macrophages which are implicated within necrotic fibres to remove damaged myofibrillar material, and (ii) $ED2^+$ macrophages which appear later, are isolated in the ECM and serve as sources for growth factors and cytokines that regulate tissue repair and regeneration (Butterfield et al., 2006; Cannon & Pierre, 1998). As with neutrophils, $ED1^+$ macrophages are suggested to contribute to inflammatory exacerbation by secreting over 100 products, such as the pro-inflammatory cytokines PGE_2 and IL-1β, in a positive feedback loop (Scott et al., 2004). Additionally, matrix metalloproteinases are released which degrade extracellular matrix components (Mackey, Donnelly, Turpeenniemi-Hujanen, & Roper, 2004). Specifically, monocyte-induced expression of matrix metalloproteinase-9 (MMP-9) is associated with the early inflammatory response (Kherif et al., 1999). Unlike neutrophils, $ED2^+$ macrophages contribute to tissue repair and regeneration in the absence of phagocytosis, with evidence that macrophage stimulation enhances regenerative processes (Lescaudron et al., 1999). This may occur due to the secretion of growth factors, cytokines and other signalling molecules such as hormones, binding proteins and ROS (Nathan, 1987). Further support is provided by research showing that macrophage depletion prevented muscle regeneration and growth in response to skeletal muscle injury (Arnold et al., 2007; Tidball & Wehling-Henricks, 2007). Such a hypothesis is consistent with the notion that the secondary response is a beneficial process in skeletal muscle repair/regeneration.

In summary, eccentric exercise poses a significant challenge to the body which results in a primary response from mechanical sources that disrupts skeletal muscle contractile integrity. Subsequent stimulation of several pathways exacerbates the response, characterised by inflammatory processes classified as the secondary response (Figure 2.1). During the latter stages of the inflammatory response and following initial phagocytosis, stimulation of repair/regeneration takes precedent. Symptoms arising from these various physiological processes encompass; impaired muscle function, increased muscle soreness and swelling, decreased range of motion and increased accumulation in the blood of markers for

intramuscular proteins, inflammatory processes and oxidative stress, which may be classified as the acute physiological responses.

2.3 Measures of Acute Physiological Responses

To determine the extent of acute physiological responses to an exercise bout, the assessment of a range of measures is common practice for investigators. Although several measures exist to directly quantify the primary and secondary response, these methods can be expensive, difficult to measure and require specific expertise. Therefore, researchers use indirect measures to quantify the magnitude of change and time course of acute physiological responses, and this section will review these measures following exercise defined by eccentric actions.

2.3.1 Muscle Function

As described in further detail in section 2.2, impaired muscle function forms part of the acute physiological response following a mechanical exercise stressor because of both primary and secondary responses. The following section will discuss methods of assessing muscle function as a measure of the acute physiological response.

2.3.1.1 Maximal Voluntary Contractions

Muscle function has typically been assessed by maximal voluntary contractions (MVC), defined as the maximal muscle force that an individual can produce under specific contractile conditions (Morton et al., 2005). MVC force is considered to be the most sensitive indirect measure of muscle function following resistance exercise (Warren, Lowe, & Armstrong, 1999) and has been suggested to orchestrate other physiological response processes (Damas, Nosaka, Libardi, Chen, & Ugrinowitsch, 2016). Eccentric exercise leads to similar decrements in force for concentric, eccentric and isometric MVC's, demonstrating impairment to the contractile machinery post-exercise (Byrne & Eston, 2002a). When individuals were clustered based on the magnitude of MVC impairment following eccentric exercise, it improved the precision of other indirect measures such as; muscle soreness, creatine kinase activity, range of motion and limb girth (Damas et al., 2016). MVC's are commonly assessed by dynamometry, and in the form of isometric or isokinetic testing have shown high reliability (correlation coefficient = 0.85-0.99) (Abernethy, Wilson, & Logan, 1995). This is despite potential limitations for this measurement which include questionable differentiation between fatigue-related reductions and stress-related reductions, the effect of an individual's motivation and the influence of investigator instruction (Warren et al., 1999). Investigators have therefore used MVC's as an assessment tool to quantify muscle function as an acute physiological response.

Studies investigating the effect of eccentric exercise on muscle function have collectively demonstrated reductions in force production post-exercise (Byrne & Eston, 2002a, 2002b; Harrison & Gaffney, 2004; Hortobágyi et al., 1998; Marginson, Rowlands, Gleeson, & Eston, 2005; Skurvydas, Brazaitis, Kamandulis, & Sipaviciene, 2010). Peak impairments regularly exceed 30% of baseline muscle force (Byrne & Eston, 2002a, 2002b; Harrison & Gaffney, 2004; Hortobágyi et al., 1998), with the magnitude of the response dependent on a variety of factors including; muscle length (Byrne, Eston, & Edwards, 2001; Child, Saxton, & Donnelly, 1998; Skurvydas et al., 2010), velocity of muscle action (Chapman, Newton, Sacco, & Nosaka, 2006; McCully & Faulkner, 1986), target muscle group (Chen, Lin, Chen, Lin, & Nosaka, 2011; Saka et al., 2009), training experience (Howatson, Van Someren, & Hortobagyi, 2007; Nosaka, Sakamoto, Newton, & Sacco, 2001) and age (Marginson et al., 2005). The magnitude of peak strength losses following eccentric actions is clear and has functional implications following exercise with regards to an individual's ability to train/compete in the acute post-exercise period.

The time course of impaired force production has been characterised by a range of studies which have displayed decrements immediately post-exercise, prior to a linear return of function over the ensuing days (Table 2.1) (Byrne & Eston, 2002a, 2002b; Harrison & Gaffney, 2004; Hortobágyi et al., 1998; Marginson et al., 2005; Skurvydas et al., 2010). A well-accepted key factor in determining the speed of recovery following eccentric exercise is prior exposure to a similar bout of exercise, whereby a second bout will report vastly reduced symptoms of stress, termed the repeated bout effect (RBE) (McHugh, 2003). Evidence also exists for a contralateral RBE whereby the impact of eccentric exercise on markers of the stress response such as creatine kinase (CK), muscle soreness and MVC was attenuated in a repeated bout of the contralateral limb (Howatson & Van Someren, 2007). The protective effect was less than that seen in the ipsilateral limb, however this study highlights an important consideration for study design when utilising crossover trials. Ebbeling and Clarkson (1990) showed that following a novel bout of eccentric arm curl exercise, participants reported a peak strength loss of 40% from initial strength immediately post-exercise which remained significantly depressed for 5 days. Upon performing a second bout of identical exercise 14 days later, despite an immediate drop in strength, muscle function had recovered within 2 days. A potential confounding factor in this study was that despite the 14-day period between exercise bouts, strength had only recovered to 87% of baseline strength at the onset of the second session. It may therefore be possible that participants were unable to exercise to a similar intensity in the second bout.

Despite being the most sensitive measure of muscle function, MVC's measured via dynamometry have been criticised for their lack of ecological validity when extrapolating to

a sporting context (Komi, 2000). Individuals engaged in resistance training in the real world may also be interested in the effect of acute physiological responses on other parameters of the force-velocity curve. Researchers have therefore assessed dynamic measures of muscle function including; vertical jumps, rate of force development and sprint performance (Byrne & Eston, 2002a; García-López et al., 2006; Highton, Twist, & Eston, 2009; Jenkins et al., 2014; Peñailillo, Blazevich, Numazawa, & Nosaka, 2015; Semark, Noakes, Gibson, & Lambert, 1999).

2.3.1.2 Jump Performance

Several acute physiological responses that occur after a mechanical exercise stimulus may impact upon maximal dynamic performance. For example, eccentric exercise is thought to preferentially impair type II fibres (Friden et al., 1983; Jones, Newham, Round, & Tolfree, 1986), which are key to the generation of power through muscular action. Damage to type II fibres results in a prolonged decrease in muscle glycogen content (Asp, Daugaard, Kristiansen, Kiens, & Richter, 1998; O'Reilly et al., 1987), which is counterproductive for explosive movements such as jumps which require high rates of energy turnover. As a result of these acute physiological responses, the power-generating ability of skeletal muscle will likely be decreased.

In relation to vertical jumps, squat jumps have been found to be affected to a greater extent compared to countermovement (CMJ) or drop (DJ) jumps following eccentric exercise (Byrne & Eston, 2002a; Harrison & Gaffney, 2004). CMJ and DJ have been proposed to benefit from potentiating mechanisms via the stretch-shortening cycle (SSC) which attenuate the negative effects of the stress response (Byrne & Eston, 2002a). Given the SSC is involved in many sporting movements, this may represent CMJ and DJ as more valid in such scenarios (Komi, 2000). Vertical jump performance has previously been shown to have high reliability (correlation coefficient > 0.97) (Aragón, 2000), however there is a lack of research quantifying this reliability across a time course commonly used to investigate the exercise-induced stress response. Following 12 sets of 10 repetitions at 60% maximal voluntary isometric contraction (MVIC) of barbell squat exercise, CMJ performance was reduced immediately post-exercise with a continued decrement at 6 h post-exercise through until a peak impairment (31%) at 24 h post-exercise (García-López et al., 2006). A limitation of this study was that CMJ performance was only measured up to 24 h post-exercise and therefore it is unknown if a continued decline would have occurred. Other research has shown similar decrements in CMJ performance, with peak impairments occurring immediately after exercise, up to 24 h post-exercise, before recovering at 72 h post-exercise (Byrne & Eston, 2002a; Harrison & Gaffney, 2004; Marginson et al., 2005). CMJ performance may be seen to be markedly impaired in the

days following resistance exercise, although further research is required to assess its sensitivity as a measure of acute physiological responses.

2.3.1.3 Sprint Performance

Sprint performance has been used as a further indirect measure to characterise acute physiological responses, as well as providing a measure of performance in the days post-exercise (Highton et al., 2009; Semark et al., 1999). Short distance sprint performance is determined by maximal acceleration (Mero, Komi, & Gregor, 1992) and has been suggested to be significantly correlated to a variety of power measures (Baker & Nance, 1999). Further to the mechanisms already proposed related to jump performance, resistance exercise has been shown to elicit significant impairments in 20 m sprint performance (Ozmen et al., 2016), which may be related to a temporary deterioration of the neuromuscular system (Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2003).

Sprint performance over 20 m has been shown to have high reliability (ICC = 0.91) (Moir, Button, Glaister, & Stone, 2004), with timing gates a more valid method of assessment compared to a global positioning system (GPS) or accelerometry (Waldron, Worsfold, Twist, & Lamb, 2011). Like vertical jump performance, further research is required to characterise the reliability of sprint performance across an applicable time scale to exercise recovery research. Following 10 sets of 10 maximal vertical jumps, 5 m and 10 m sprint performance were impaired at 24 h (5%) and 48 h post-exercise (6% and 5%, respectively) (Highton et al., 2009). In contrast, no change in sprint performance over 5 m, 10 m, 20 m and 30 m following 70 drop jumps has been reported (Semark et al., 1999). The discrepancy may be related to the magnitude of the physiological response whereby Semark et al. (1999) demonstrated no change in CK throughout the study, suggesting the exercise bout was not sufficient to disrupt membrane integrity. Together these studies suggest that sprint performance may be impaired following sufficiently strenuous eccentric exercise. Further research investigating the effect of exercise protocols reflective of real-life practice on sprint performance will help to ascertain whether impairments are ecologically valid. Measures of sprint performance may therefore allow the assessment of muscle function, reflecting several acute physiological responses.

2.3.1.4 Rate of Force Development

Given MVC's may be confounded by the effects of muscle fatigue, investigators have explored alternative measures of muscle function. The assessment of rate of force development (RFD) has proven promising with evidence that different time intervals can differentiate the mechanisms of force loss following eccentric exercise. Recent reports suggest that RFD calculated during the early phase (0-100 ms) of the contraction may be a sensitive indicator of force loss associated with neuromuscular function (Jenkins et al., 2014), while

later phase intervals (100-200 ms) differentiate muscle damage from fatigue (Peñailillo et al., 2015).

Research has previously reported the typical error for RFD between 100-200 ms to be within 5-11% (Buckthorpe, Hannah, Pain, & Folland, 2012; Tillin, Pain, & Folland, 2013). To date, there is no evidence to characterise the reliability of RFD across a time scale that is common to exercise recovery research. Jenkins et al. (2014) showed that following 6 sets of 10 maximal eccentric actions, RFD between 0-200 ms was impaired for up to 72 h post-exercise, with a peak decrement of 52% immediately post-exercise. The authors suggested that RFD from 0-200 ms followed a similar time course to that of MVC strength losses, whereas RFD calculated from earlier time points (e.g. 0-10, 0-50 and 0-100 ms) had recovered by 72 h post-exercise and were therefore more reflective of neural mechanisms. Peñailillo et al. (2015) reported that RFD from 100-200 ms was decreased ~30% immediately following a bout of eccentric cycling, however there was no change following concentric cycling.

RFD is also dependent upon the type of muscle action, with concentric actions the most favourable for explosive performance, due to greater neural activation (Tillin, Pain, & Folland, 2012). Despite this, most reports have investigated RFD related to isometric muscle actions (Jenkins et al., 2014; Peñailillo et al., 2015). To enhance the ecological validity of RFD in research related to acute physiological responses, further insights are required to characterise post-exercise changes across time intervals using a mode of exercise defined by concentric muscle actions.

In summary, a bout of resistance exercise stimulates acute physiological responses resulting in impaired muscle function that may be measured by MVC's, jump and sprint performance as well as RFD. These measures represent different aspects of the force-velocity curve (e.g. peak force, maximal force generation and indices of power and acceleration) and therefore may be included concomitantly in studies to provide a holistic picture of muscle function, whilst enhancing ecological validity. Decrements in muscle function typically manifest immediately post-exercise and recover over the ensuing days, which may negatively impact upon exercise training/performance during this period. Although the magnitude of change and time course of muscle function as an acute physiological response has been suggested, further insights are required to characterise the sensitivity of these measures to detect change that is real. This will also aid our ability to determine if changes resulting from interventions are great enough to be meaningful. Given the widespread use of resistance exercise across a variety of sports, an understanding of its effects on a range of aspects of muscular performance is warranted to optimise training programming and recovery processes.

Table 2.1 Overview of acute physiological response measures.

		Time post-exercise					
Acute physiological response	Measure	2 h	6 h	24h	48 h	72 h	96 h
Muscle function	MVIC	↓↓↓	↓↓↓	↓↓↓	↓↓	↓	↓
	Jump performance	↓↓	↓↓	↓↓↓	↓↓	↓	↓
	Sprint performance			↓↓	↓↓		
	RFD	↓↓↓	↓↓↓	↓↓↓	↓↓	↓	
Muscle soreness	VAS			↑↑↑	↑↑↑	↑↑	↑
Intramuscular proteins	Creatine kinase			↑↑	↑↑	↑↑↑	↑↑↑
Inflammation	IL-6		↑↑	↑			
	IL-10		↑↑				
	CRP			↑↑	↑↑		
	MMP-9	?	?				↑↑
Oxidative stress	Protein carbonyls			↑↑	↑↑		
	LOOH	↑					
	Ascorbyl free radical	?	?	?	?		
Others	Limb girth			↑↑	↑↑	↑	↑
	Range of motion			↑↑	↑↑		
	Sleep			?	?	?	?
	DALDA	?	?	?	?	?	?

MVIC, maximal voluntary isometric contraction; RFD, rate of force development; VAS, visual analogue scale; IL-6, interleukin-6; IL-10, interleukin-10; CRP, C-reactive protein; MMP-9, matrix metalloproteinase-9; LOOH, lipid hydroperoxides; DALDA, daily analysis of life demands for athletes. One arrow, minor increase/decrease; two arrows, moderate increase/decrease; three arrows, large increase/decrease. Question mark indicates a potentially affected time point in the absence of evidence.

2.3.2 Muscle Soreness

Section 2.2 described the mechanisms purported to underpin the acute physiological responses following eccentric actions, however despite its association, the precise cause of muscle soreness with this type of exercise is yet to be fully elucidated. Given that muscle soreness is a common outcome of performing resistance exercise, it was traditionally speculated that muscle soreness arises through muscle and/or connective tissue damage (akin to the popping sarcomere hypothesis), and/or the subsequent inflammatory cascade caused by the secondary response (Armstrong et al., 1991; Clarkson & Hubal, 2002; Nosaka, Newton, & Sacco, 2002). Connective tissue damage as a cause is feasible due to muscle pain receptors which are most concentrated in tendons and connective tissue (Newham, 1988), while oedema and the breakdown products of injured tissue that accompanies the local inflammatory response could sensitise nociceptors (Proske & Morgan, 2001). However a causal link has not been confirmed for either of these suppositions (Cleak & Eston, 1992; Malm et al., 2000). Despite this, due to its prevalence, muscle soreness is still an important facet of the acute physiological response following resistance exercise.

In an exercise setting, pain has been used interchangeably with soreness to describe muscular discomfort associated with the days post-exercise (Nosaka et al., 2002). Investigators have often used rating scales to determine the intensity of post-exercise muscle soreness (MacIntyre, Reid, & McKenzie, 1995), with good reliability of visual analogue scales reported within acute clinical pain research (intraclass correlation coefficient of 0.96) (Bijur, Silver, & Gallagher, 2001). However, there is a lack of evidence to provide information as to the reliability of visual analogue scales in the setting and time scale of exercise recovery.

To assess whether muscle soreness reflected the magnitude of muscle damage following eccentric exercise, Nosaka et al. (2002) had 110 participants complete either 12 (n = 50) or 24 (n = 60) maximal eccentric actions of the elbow flexors. Ratings of muscle soreness on a 50 mm visual analogue scale were elevated 24 h, prior to a peak at 48 h post-exercise (40 mm). There was a linear recovery from 48 h to 72 h post-exercise, although muscle soreness was still elevated above baseline. The magnitude and time course of muscle soreness shown by Nosaka et al. (2002) is consistent with other reports following eccentric exercise (Table 2.1) (Brown, Child, Donnelly, Saxton, & Day, 1996; Lee et al., 2002; Malm et al., 2000; Vincent & Vincent, 1997b). Despite this muscle soreness response, it was found that there were no differences between individuals that had performed either 12 or 24 eccentric actions nor were there any correlations between muscle soreness and other criterion measures such as MVC, range of motion (ROM), limb girth and CK activity (Nosaka et al., 2002). In spite of the authors concluding that muscle soreness in the days post-exercise does not reflect the magnitude of the acute physiological response, given that the mechanisms of muscle soreness

are yet to be elucidated, it may be possible that in fact muscle soreness reflects a valuable additional aspect (Cheung, Hume, & Maxwell, 2003). A possible limitation of muscle soreness assessed via a visual analogue scale is that it only refers to the intensity of the soreness and not how the individual reacts to the soreness (MacIntyre et al., 1995). Vincent and Vincent (1997b) have shown that ratings of muscle soreness are similar between trained and untrained individuals, also supported by others (Newton, Morgan, Sacco, Chapman, & Nosaka, 2008). Given that other aspects of the post-exercise stress response are attenuated with repeated bouts (Nosaka et al., 2001), although speculative, it may be possible that trained individuals are less affected by a similar intensity of soreness to function at a greater level following strenuous exercise.

Although muscle soreness may not reflect the magnitude or time course of other markers of the stress response, it is clearly implicated as a physiological response in the days post-exercise. Visual analogue scales provide a convenient, non-invasive means of assessing an individual's perception of the level of soreness imposed by eccentric exercise, however further research is required to provide information as to its sensitivity as a measurement in an exercise setting. Given the prevalence of muscle soreness with eccentric exercise which is generally seen as a negative consequence, being able to quantify these perceptions appropriately is key for investigators that aim to introduce strategies to reduce post-exercise muscle soreness for the benefit of athletic individuals and recreational exercisers alike.

2.3.3 Intramuscular Proteins & Enzymes

Several intramuscular proteins and enzymes have been identified as indirect markers of the exercise-induced physiological response. Appearance of such proteins/enzymes including; CK, lactate dehydrogenase (LDH) and myoglobin, may indicate tissue disruption when concentrations exceed those of homeostatic conditions (Brancaccio, Maffulli, & Limongelli, 2007). Increased permeability of muscle cell membranes following the primary response allows 'leakage' of cellular proteins into extracellular fluid and blood which is prevalent for several days post-exercise (Brancaccio et al., 2007; Howatson & Van Someren, 2008).

In relation to these substances, CK has been the most widely used, possibly due to both a large post-exercise accumulation and relatively modest assay cost in comparison to other proteins and enzymes (Clarkson & Hubal, 2002). Creatine kinase plays a key role in muscular contraction as it catalyses the reversible exchange of high energy phosphate bonds between phosphorylcreatine and adenosine diphosphate (ADP) to buffer cellular adenosine triphosphate (ATP) concentrations (Brancaccio et al., 2007). Creatine kinase exists in three cytosolic isoforms which are located in the brain (CK-BB), as well as skeletal (CK-MM) and cardiac (CK-MB) muscle (Koch, Pereira, & Machado, 2014). CK-MM is the isoform that acts

as a marker of exercise-induced stress and its accumulation in the blood may therefore be reflective of muscle cell disruption (Koch et al., 2014). CK could also have immune-modulatory actions, serving as a messenger molecule between skeletal muscle and the immune system (Malm, 2002).

Creatine kinase has been reported to have moderate test-retest reliability ($r = 0.38$) in response to 120 minutes of simulated soccer match play, however with a coefficient of variation (CV) of 28%, this highlights potential variability with this measure (Harper et al., 2016). Large inter-individual variability is part of the criticism for CK as a reliable marker to describe exercise-induced stress (Clarkson & Ebbeling, 1988). Analysis of 483 male and 245 female athletes found that individuals from sports that included eccentric actions (e.g. football players) demonstrated higher levels of CK compared to those performing concentric contractions during non-weight bearing activities (e.g. swimmers) (Mougios, 2007). Additionally, compared to a cohort of 137 male and female non-athletes, those currently participating in sport had higher levels of CK. Miles et al. (2008) found that during a control week, despite a large CV (54%), between-day CK values were within a similar range (144-153 IU/L^{-1}). Although the full cause of CK inter-individual variability is yet to be elucidated (Clarkson & Hubal, 2002), together these results demonstrate the need to compare changes in CK between homogenous groups that are matched for training status and type of activity performed, with comparisons made to a time matched control condition to account for inter-individual differences.

Creatine kinase concentrations in the blood have consistently been shown to be elevated following exercise that incorporates an eccentric component (Table 2.1) (Clarkson & Hubal, 2002), and log CK has been suggested to have a close relationship with work decrease in an eccentric exercise bout (Hody, Rogister, Leprince, Wang, & Croisier, 2013). Following 24 maximal eccentric actions of the elbow flexors, CK concentrations were elevated at 48 h through to 168 h post-exercise, with peak increases (mean peak = 12,872 IU/L^{-1}) occurring at 72-96 h post-exercise (Nosaka & Clarkson, 1996). Individuals with the greatest peak in CK were also found to have a more profound abnormality in magnetic resonance imaging, supporting the magnitude of CK response as an indirect marker of mechanical alterations in skeletal muscle. The magnitude and time course of the CK response to eccentric exercise has similarly been reported by others (Clarkson, Nosaka, & Braun, 1992; Miles et al., 2008; Nosaka, Chapman, Newton, & Sacco, 2006; Nosaka & Clarkson, 1996; Rodrigues et al., 2010). In contrast, Vincent and Vincent (1997b) found the range of peak concentrations to be 1200-6100 and 125-1500 IU/L^{-1} for untrained and trained individuals respectively in response to a lower-limb resistance exercise session. Creatine kinase values appear to rise to a greater extent following upper body exercise than that of the lower limbs. Given trained individuals

displayed a smaller magnitude of peak CK response compared to untrained counterparts (Vincent & Vincent, 1997b), previous exposure to eccentric exercise may be a key determinant of the response. This may explain the differences between upper and lower limbs whereby lower limbs are habituated with eccentric loading during daily activities such as walking and descending stairs.

It has been reported that the magnitude of change and time course of the response following eccentric exercise will vary depending on the intramuscular protein or enzyme in question (Nosaka & Clarkson, 1996). CK, LDH and myoglobin are cytosolic proteins, and are therefore reflective of damage to the sarcolemma. Structurally bound proteins such as myosin heavy chains and troponin have also been investigated in relation to the primary response following eccentric exercise, albeit to a lesser extent, and may more closely reflect muscle function (Sorichter et al., 1997). Rankin, Stevenson, and Cockburn (2015) found that following an eccentric exercise protocol designed to elicit muscle damage, both males and females saw a peak rise in the concentration of both CK and skeletal troponin at 72 h post-exercise. This was also the last sampling point and therefore it is unknown if there would have been a continual rise after this time. Although the inter-individual variability of the response seen for troponin was less than that for CK, the magnitude of change was greater for CK. Further research characterising both cytosolic and structural proteins following real world exercise sessions and across a longer timescale will aid our understanding of the primary response, whilst also identifying the most sensitive measures to define acute physiological responses following resistance exercise.

Intramuscular proteins and enzymes as an indirect marker of the stress response do not necessarily correlate with changes in muscle function (Warren et al., 1999), however change in the blood concentration for these measures may reflect aspects of the primary response. Caution must be used with the interpretation of analysis as blood concentration is a reflection of what is being produced in the muscle and what is being cleared from the blood at a given point in time (Clarkson & Hubal, 2002), therefore an understanding of the recommended sampling points to capture the peak change is key. An appreciation of the confounding factors associated with these measures allows investigators to make appropriate interpretations as to whether the changes are worthwhile signals amongst the noise of inter-individual variability. Being able to characterise measures that reflect the primary response allows practitioners to gain an insight into the physiological load of an exercise session for the purposes of monitoring training.

2.3.4 Inflammation

As discussed in section 2.2, inflammatory processes that form part of the acute physiological response is regulated by the secondary response which may be activated by calpain, increased Ca^{2+} concentration, perturbation of muscle fibres or sensing of damage by local macrophages (Butterfield et al., 2006). To provide a measure of inflammation, investigators have used cytokines in the blood which regulate inflammatory processes as a proxy for what is occurring within the muscle (Suzuki et al., 2003). This method is not without limitation as there is sometimes a dissociation between local gene expression in skeletal muscle and the systemic concentration of cytokines (Peake, Della Gatta, Suzuki, & Nieman, 2015). Blood sampling is less invasive than the muscle biopsy procedure, with analytical techniques also easier, which allows the comparison of responses in different studies (Peake, Nosaka, & Suzuki, 2005). Therefore, using cytokines in the blood as a measure, offers an indirect view of inflammatory processes as local and systemic mediators of repair and regeneration.

Serum IL-6 is one of the most studied cytokines, due to its large magnitude of change following strenuous exercise (Peake, Suzuki, et al., 2005) and is thought to be the main systemic mediate of the acute phase response following exercise (Pyne, 1994). Although consistent in its appearance, the type of muscle action impacts upon the time course of systemic IL-6 production, with a later appearance following eccentric actions in comparison to endurance exercise possibly owing to its role in the secondary response as opposed to metabolic processes (Febbraio & Pedersen, 2002). Following eccentric exercise, IL-6 has been shown to peak 8 h and return to baseline by 24 h post-exercise (Miles et al., 2008), which is consistent with intramuscular IL-6 expression which peaked 8-12 h post-exercise (Tomiya, Aizawa, Nagatomi, Sensui, & Kokubun, 2004). Previous research has reported a diurnal variation associated with IL-6 production (Miles et al., 2008) and therefore future investigations are recommended to control for time of day as well as utilise a control condition in experimental designs. If these factors are considered, serum IL-6 concentrations provide a useful surrogate marker of inflammatory processes related to the secondary response.

Although IL-6 has typically been viewed as a pro-inflammatory cytokine, there is evidence that it is also involved in anti-inflammatory cascades (Reihmane & Dela, 2014; Steensberg, Fischer, Keller, Møller, & Pedersen, 2003). This is dependent upon the type of signalling, with classic signalling referring to IL-6 binding with its cell membrane bound receptor (IL-6R), exerting an anti-inflammatory effect (Reihmane & Dela, 2014). While trans-signalling refers to IL-6 forming a binary complex with the soluble form of IL-6R (sIL-6R), exerting a pro-inflammatory effect (Muñoz-Cánoves, Scheele, Pedersen, & Serrano, 2013). Robson-Ansley, Cockburn, Walshe, Stevenson, and Nimmo (2010) suggested that there is an association between sIL-6R, perception of pain and reduced muscle function following eccentric exercise,

supporting its role in the acute physiological response, although the authors conclude that further work is required to explore this relationship. Further down the IL-6 mediated anti- and pro-inflammatory cascades is the stimulation of IL-10 (Steensberg et al., 2003) and C-reactive protein (CRP) (Petersen & Pedersen, 2005), respectively.

IL-10 is thought to induce several anti-inflammatory actions, primarily influencing the inhibition of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-8 (Moore, O'garra, Malefyt, Vieira, & Mosmann, 1993). The anti-inflammatory role of IL-10 may serve an important function as part of the inflammatory cascade within skeletal muscle, ensuring tissue repair (Malm, 2002). As with IL-6, IL-10 production post-exercise is dependent upon the mode of exercise with factors other than acute physiological responses causing cytokine production following endurance exercise. These include energy availability, metabolic and hormonal alterations which are known as stimuli for systemic cytokine release (Suzuki et al., 2003). Hirose et al. (2004) reported that in untrained males performing two bouts of eccentric exercise, separated by four weeks, IL-10 production increased at 6 h post-exercise only after the second bout. The authors attributed this to the role of IL-10 in reducing inflammation and promoting adaptation in the subsequent exercise bout. Whether this response of IL-10 would be seen in a trained cohort or following a period of resistance training reflective of a real-world protocol is yet to be determined.

IL-6 is a systemic precursor to hepatic CRP production (Donges, Duffield, & Drinkwater, 2010) and chronically elevated concentrations have been implicated in the process of aging as well as a risk factor for several health conditions (Olson, Dengel, Leon, & Schmitz, 2007; Stewart et al., 2007). When viewed in the context of exercise, CRP forms part of the acute-phase inflammatory response (Kasapis & Thompson, 2005). Following eccentric exercise, CRP concentrations have been reported to peak at 24-48 h post-exercise (Dousset et al., 2007; Malm et al., 2000). Other measures of acute physiological responses such as muscle soreness/swelling have been seen to have low association with the initial inflammatory cytokines and may be more reflective of the secondary response driven by inflammatory processes such as CRP (Miles et al., 2008). In contrast to IL-10, CRP concentrations are seen to be attenuated with regular exercise which may highlight a more efficient inflammatory response with increasing training status (Donges et al., 2010). If the inflammatory response could be manipulated via strategies employed in the acute post-exercise period, this may be of benefit for long-term adaptation, however further research is required to investigate this.

Matrix metalloproteinases (MMPs) are secreted proteinases that serve to degrade the extracellular matrix as part of the adaptive process following strenuous exercise (Sternlicht & Werb, 2001). Expression of several MMPs, specifically MMP-9, is of interest following

eccentric exercise due to its production by a variety of leukocytes and its association with the early inflammatory response (Mackey et al., 2004). Whilst it has been reported that circulating MMP concentrations are indicative of changes at the tissue level (Kasahara et al., 1997). Mackey et al. (2004) reported that serum MMP-9 concentrations were elevated 8 days following a bout of eccentric exercise, however others have shown no effect (Koskinen et al., 2001; Madden, Byrnes, Lebin, Batliner, & Allen, 2011). Aside from immediately post-exercise, the earliest sampling point used in these studies was 24 h post-exercise. Given that MMP-9 is suggested to be associated with the early inflammatory response, it would be prudent for future investigations to include sampling points that coincide with this period (e.g. 2-8 h post-exercise). Urso, Pierce, Alemany, Harman, and Nindl (2009) reported that the transient increase in MMP-9 following eccentric exercise was maintained in a second bout of exercise after 8 weeks of training. However, the peak concentration occurred earlier which the authors attributed to activation of the MMP system which is likely a critical component of skeletal muscle adaptation and the initiation of remodelling cascades.

Taken together, IL-6, IL-10, CRP and MMP-9 offer insights as measures of inflammatory processes related to the acute physiological response. Inflammation, as the cellular response of the organ to strenuous exercise, is essential for proper tissue repair and, in the case of skeletal muscle, complete functional regeneration (Chazaud, 2016). Future research should consider possible diurnal variation and employ a time-matched control comparator as well as sampling points to capture changes in the acute-phase inflammatory response. It is also of interest to further investigate the role of exercise training in modulating the inflammatory response whilst considering the possibility of strategies that may manipulate these processes in the acute post-exercise period for the purposes of recovery and/or adaptation.

2.3.5 Oxidative Stress

As discussed in section 2.2, free radicals and ROS are produced during the secondary response following eccentric exercise and are typically released by neutrophils to serve a phagocytic function (Scott et al., 2004). Exposure to free radicals and ROS can harm nearby uninjured cells, also exacerbating the primary response (Close et al., 2005). Often used interchangeably, free radicals and ROS are two separate classes of molecule (Lushchak, 2014). Free radicals have one or more sets of unpaired electrons, whereas ROS are have fully paired electrons but are highly reactive (Halliwell, 2007). Unless specified, the term ‘ROS’ will be used to also incorporate free radicals. The term ‘oxidative stress’ may therefore refer to a disrupted redox homeostasis that underpins oxidative damage (e.g. lipid, protein and deoxyribonucleic acid [DNA] oxidation) and/or redox signalling (Jones, 2008; Sies, 1985, 2015). Measurement of oxidative stress as an acute physiological response is particularly difficult due to: markers lacking exclusivity to oxidative damage, oxidised macromolecule adducts are subject to

repair, global levels of macromolecule adducts are not reflective of redox signalling and/or damage to other macromolecules, optimal sampling time is heterogenous, and redox biomarkers exhibit circadian oscillation (Cobley, Close, Bailey, & Davison, 2017). Therefore investigators are recommended to use multiple markers to assess and support the presence of oxidative stress (Cobley et al., 2017).

Lipid hydroperoxides (LOOH) are an oxidised molecule that can be used to identify lipid peroxidation by ROS (Alessio et al., 2000). LOOH can be reliably quantified in the blood using the spectrophotometric method based on ferrous oxidation of xylenol orange 1 (FOX1) (Wolff, 1994). Several studies have shown LOOH to increase following isometric exercise (Alessio et al., 2000), aerobic exercise (Alessio et al., 2000; Fogarty et al., 2011) and in disease populations (Davison et al., 2002). In contrast, Deminice, Sicchieri, Payao, and Jordao (2010) reported no change in LOOH when assessed 10 min following a resistance exercise session. Given the importance of sampling time in the assessment of oxidative stress, it may have been that changes in LOOH were not captured in this study. Further research is required to characterise the production of LOOH following resistance exercise to quantify this molecule as a measure of the secondary response.

Oxidation of proteins can also be quantified by the analysis of the presence of carbonyl groups (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003). Protein carbonyls (PC) have been regularly shown to increase following eccentric exercise although this method is unable to detect which proteins per se are oxidised and so provides a global measure of protein oxidation (Nikolaidis et al., 2008). Several studies have shown that the concentration of PC in the blood increases following eccentric exercise with concentrations peaking at 24-48 h post-exercise (Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Goldfarb, Bloomer, & Mckenzie, 2005). Bowtell et al. (2011) reported that the post-exercise increase in PC was attenuated when participants consumed an antioxidant supplement (Montmorency tart cherry juice) which coincided with an accelerated recovery of muscle strength. PC may therefore provide a measure of oxidative stress which links with other acute physiological responses. Future research may include this measure to determine strategies which can manipulate acute physiological responses for enhanced exercise recovery.

Developments in analytical techniques have made it possible to directly measure free radical production. Plasma levels of the lipid-derived ascorbyl free radical are produced via oxidation of ascorbate and have been used as a measure of oxidative stress in both exercise and clinical settings (Ashton et al., 1998; Buettner & Jurkiewicz, 1993; Pietri, Séguin, d'Arbigny, & Culcasi, 1994). Research has demonstrated exhaustive aerobic exercise to increase ascorbyl free radical production (Davison, Ashton, Davies, & Bailey, 2008; Davison et al., 2002),

however levels were unchanged following a bout of repeated sprint exercise (Clifford et al., 2016). Differences in the training status of participants may explain some of these differences, however, further research is also required to investigate the effect of resistance exercise on ascorbyl free radical production.

The measurement of oxidative stress *in vivo* comes with its challenges and investigators are recommended to use more than one marker and to use markers that both directly and indirectly quantify lipid and protein oxidation to provide a more informed view of oxidative stress. Given the biochemistry of ROS, understanding the optimal sampling time is key, while further research into the manipulation of these measures as an acute physiological response with post-exercise strategies will also provide a valuable addition to the literature.

2.3.6 Other Markers

There are several other methods of assessment that are used to either give indirect information about acute physiological responses (e.g. limb girth/ROM) or to quantify the effect of these responses on other aspects of the individual (e.g. sources of stress/sleep analysis). Measures that an individual may be more consciously aware of are important as the subjective perception of pain and readiness for exercise can enhance exercise recovery (Broatch, Petersen, & Bishop, 2014).

Both limb girth and ROM have been used as surrogate markers for oedema and inflammation, particularly due to their ease of use, non-invasive nature and relative cost to implement. Oedema formation has been attributed to increased intra- and extravascular fluid content (Schnizer, Hinneberg, Moser, & Küper, 1979) because of blood vessel haemorrhaging (Knight, 1995) and a raised osmotic gradient following cellular accumulation of metabolites (Bobbert, Hollander, & Huijing, 1986). The limitation of these methods is the poor inter-rater reliability, however, good intra-rater reliability has been shown and it is therefore recommended that the same investigator conduct the measure throughout for comparison (Warren et al., 1999). Several reports have demonstrated increases in limb girth and ROM following eccentric exercise, which have typically peaked 24-48 h post-exercise (Chen et al., 2011; Howatson & Milak, 2009; MacDonald, Button, Drinkwater, & Behm, 2014), although others have suggested the effects to last for up to 10 days post-exercise (Clarkson & Hubal, 2002). Limb girth and ROM may be used as surrogate markers for oedema and the secondary response and can provide a useful tool for practitioners in the field. Further research to identify the optimal sampling point and the characterisation of this time course alongside established measures of inflammation (e.g. circulating levels of cytokines) will enable a more informed use.

Daily monitoring of an athlete is commonplace for coaches in an attempt to minimise the risk of overtraining which can manifest in a range of symptoms including psychological disturbances and disruption to sleep (Meeusen et al., 2006). The daily analysis of life demands for athletes (DALDA) questionnaire was devised by Rushall (1990) and encapsulates both the sources of stress and the manifestation of stress as symptoms. Previous research has demonstrated the DALDA to be sensitive to distinguish overreaching athletes during a period of intensified training (Coutts, Slattery, & Wallace, 2007; Halson et al., 2002) and throughout a competitive season (Nicholls, Backhouse, Polman, & McKenna, 2009), as well as differentiate the efficacy of recovery strategies following a bout of strenuous exercise (Wilson et al., 2018).

Advancement in the technology of activity monitors has enabled sleep monitoring in real-world environments, while established guidelines have allowed this technique to be validated against the gold-standard polysomnography (Sargent, Lastella, Halson, & Roach, 2016). It has been suggested that elite athletes suffer from poorer characteristics of sleep compared non-athletes which may be related to a high volume/intensity of exercise (Leeder, Glaister, Pizzoferro, Dawson, & Pedlar, 2012). Sleep is also affected depending on the type of sport an athlete engages in, with individual sports suffering poorer sleep characteristics than team sports (Lastella, Roach, Halson, & Sargent, 2015). No studies have investigated the DALDA or sleep in relation to an acute bout of resistance exercise and this would therefore be a useful addition to the literature to characterise the usefulness of these measures in this context.

To provide a holistic picture of acute physiological responses, it is important to consider how these may impact an individual's perception on their readiness to exercise. Research that provides insights into the magnitude of change and time course of measures that may be readily used in the real-world will enable practitioners to optimise sampling time, understand the sensitivity of these in relation to a bout of resistance exercise and determine the effectiveness of recovery strategies.

2.4 Recovery/Adaptation Paradigm

The primary focus of this thesis to date has been on the acute physiological responses in the hours and days following a single bout of resistance exercise. What is clear is that some of these responses cause deleterious consequences to performance that investigators/practitioners have attempted to combat through the application of recovery strategies. In the context of training, especially related to resistance exercise, the goal is directed towards physiological adaptation. In recent years, there is growing evidence that manipulating the acute physiological responses post-exercise may influence training adaptation. The following section will discuss this dichotomy.

2.4.1 Hormesis

It has been proposed that exercise creates a system that resembles hormesis, whereby hormesis relates to biological systems responding to homeostatic perturbations in a bell-shaped curve (Radak et al., 2008). This theory which is derived from toxicology, suggests that hormesis is characterised by low-dose stimulation and high-dose inhibition and was first translated into the field of exercise physiology by Radak, Chung, and Goto (2005). These authors proposed that under normal conditions, the "toxin" in this case for example inflammation/oxidative stress, evokes specific and systemic adaptations that allow the body to better cope with subsequent exercise challenges (Radak et al., 2008). At the other end of the hormesis curve, exhaustive exercise or overtraining can overwhelm the body's ability to recover and adapt, leading to maladaptation (Ogonovszky et al., 2005).

2.4.2 Adaptation

To apply the concept of hormesis to the recovery/adaptation paradigm, it is necessary to understand the process by which the transient perturbations to homeostasis (associated with acute physiological responses), translate to adaptation as part of a training programme. Many features of the exercise-induced adaptive response and the ensuing signalling mechanisms are specific to the type of exercise (Coffey & Hawley, 2007). Therefore, the following section will focus on adaptations to resistance exercise. Although a full review of this process is outside the scope of this thesis, the role of acute physiological responses in this cascade will be reviewed.

As discussed in section 2.2, resistance exercise initiates a sequence of events that have been categorised into a primary and secondary phase. Although this may result in short-term deleterious consequences, these acute physiological responses are suggested to be required for beneficial longer-term adaptations, of which inflammation and oxidative stress are the primary sources (Schoenfeld, 2012).

A large body of evidence exists to suggest that macrophages play an important role in the repair and regeneration of skeletal muscle following resistance exercise (Tidball, 2005). Some of the key cytokines that contribute to the hypertrophic response include IL-6 and IL-10 (Schoenfeld, 2012), which are secreted by macrophages. IL-6 has been proposed as an essential regulator of satellite cell-mediated hypertophic muscle growth (Serrano, Baeza-Raja, Perdiguero, Jardí, & Muñoz-Cánoves, 2008). These authors demonstrated that IL-6 stimulated the proliferative potential of myoblasts *in vitro*, whilst in the absence of IL-6 (although satellite cells could be normally activated), they were incapable of proliferating and differentiating at subsequent stages of the muscle growth process. The timing of resolution of inflammation is also important for the repair/regeneration of skeletal muscle, to which IL-10 plays a key role

(Chazaud, 2016). Both early initiation or secondary phase inhibition of the anti-inflammatory response, via IL-10 injections or blockage of anti-IL-10 antibodies, respectively, impairs muscle regeneration (Hunt, Upadhyay, Jazayeri, Tudor, & White, 2013; Perdiguero et al., 2011). The link between the inflammatory response and resistance training adaptation is further supported by the finding that lower circulating CRP levels are correlated with increased strength gains following an exercise intervention in sedentary populations (Donges et al., 2010; Olson et al., 2007). Inflammation is an acute physiological response that is mandatory for the tissue repair and function regeneration that form part of the adaptive response in skeletal muscle following resistance training (Chazaud, 2016).

Neutrophils, released during the secondary response following eccentric exercise, may also play a role in adaptation as they are responsible for the production of ROS which can function as key cellular signalling molecules (Schoenfeld, 2012). ROS have been proposed to stimulate a variety of hypertrophic pathways including: mitogen-activated protein-kinase (MAPK) (Kefaloyianni, Gaitanaki, & Beis, 2006) and insulin-like growth factor (IGF-1) signalling (Handayaningsih et al., 2011). Further support for the adaptive role of ROS comes from research demonstrating suppressed training responses with the prevention of exercise-induced redox signalling via antioxidant supplementation (Powers, Duarte, Kavazis, & Talbert, 2010), which is discussed in section 2.4.4.

Beneficial adaptations to resistance exercise have been shown to occur without exercise-induced muscle damage *per se* (Malm & Yu, 2012) and may relate more specifically to a sufficient stimulus promoted by the acute physiological responses within the concept of hormesis. It is important to acknowledge that the anabolic signalling pathways activated by inflammation and/or oxidative stress may also be triggered by other stimulatory factors, which are outside the scope of this thesis, and as such there is a level of redundancy within the hypertrophic response. Further research is required to provide further insights into the role of acute physiological responses on adaptation to resistance training.

2.4.3 Other Adaptations to Resistance Exercise

Although a full review of the proposed adaptations to resistance exercise is outside the scope of this thesis, it is important to acknowledge that there are several ways in which the human phenotype adapts in response to resistance exercise. In keeping with the applied nature of this thesis, it is also essential to consider the end goal for individuals engaging in regular resistance exercise. To that end, the following section will review the determinants of strength and other adaptations to resistance training.

A wealth of research has pinpointed that the primary determinants of increases in strength following resistance training are as a result of neuromuscular adaptations, increases in muscle

size, and alterations in connective tissue stiffness (Knuttgen & Kraemer, 1987). Following the onset of training, individuals can be expected to rapidly gain strength via a learning effect in which the individual is able to more efficiently recruit a greater proportion of muscle fibres with reduced agonist activation, as well as eliciting connective tissue adaptations (Sale, 1988). After this initial adaptation has occurred, progression slows, and the focus turns towards increasing muscle size, typically due to muscle protein synthesis which is derived from the stimulation of molecular pathways (Hughes, Ellefsen, & Baar, 2018). With prolonged strength training individuals reach a plateau with only small changes demonstrable and must incorporate new stimuli to progress strength (Hughes et al., 2018).

Often used as the gold-standard for lower body strength, back squat performance has been reported to be influenced by psychological, anthropometric and biomechanical factors (Vigotsky et al., 2018). In this study, it was found that squat strength is primarily predicted by anthropometric variables of which fat-free mass relative to height was deemed to be most important. In the unlikely scenario of altering the height of a fully matured adult, this study suggests that increasing skeletal muscle mass (here determined by fat-free mass), may be the most promising route to deliver increased lower body strength. However an alternative viewpoint suggests that strength is not necessarily indicative of adaptations accrued through regular resistance exercise and therefore cannot always determine an individual's training status (Buckner et al., 2017).

A bulk of research has focused on neuromuscular adaptations to resistance training due to the speed in which these changes become apparent (Hughes et al., 2018). Neuromuscular adaptations may be considered to arise through either increases in skill acquisition via the nervous system or increased maximal muscle activation by greater motor unit synchronisation, muscle recruitment and neural activation (Hortobagyi & Maffiuletti, 2011; Jones, Rutherford, & Parker, 1989). Perhaps the most rudimentary evidence of the role of neuromuscular adaptations in the development of strength is by way of cross-education. Cross-education is the performance improvement (e.g. strength) of the untrained limb following a period of unilateral practice (e.g. strength training) of the homologous contralateral limb and may be attributed to neural mechanisms as it occurs in the absence of muscle hypertrophy (Farthing, Borowsky, Chilibeck, Binsted, & Sarty, 2007). Although the precise mechanisms underpinning this phenomenon are still to be fully elucidated, suggestions that localised muscular adaptations, cross-limb cortical interaction, and adaptations to spinal cord excitability may each play a role (Carroll, Herbert, Munn, Lee, & Gandevia, 2006). The role for changes in brain activation in cross-education has been supported by Farthing et al. (2007) who found that after training, there was an enlarged region activation in contralateral sensorimotor cortex and left temporal lobe during muscle contractions with the untrained arm.

The transfer of strength has been shown to be greater when the training load is eccentric versus concentric (Kidgell et al., 2015) and cross-education may also be further augmented when the exercising limb is viewed in a mirror (Zult et al., 2016). Despite consensus on the mechanism remaining open, neuromuscular adaptations clearly play a key role in the early phase of strength development.

An additional output from neuromuscular adaptations induced by strength training is an increased RFD (Maffiuletti et al., 2016). Research has demonstrated a 15% increase in RFD following resistance training in which there was a concomitant increase in both electromyography amplitude and rate of electromyography increase, indicating an enhancement in neural drive (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002). Other factors may also contribute to increases in RFD including muscle fibre type and force transfer via the cytoskeletal network (Hughes et al., 2018), which highlights the complexity with which adaptations occur and the way in which seemingly isolated aspects may be interlinked.

It is well-established that resistance exercise is a potent stimulus for muscle protein synthesis which results in an increase in skeletal muscle mass (Damas, Phillips, Vechin, & Ugrinowitsch, 2015). The most important signal for muscle growth in an adult is the load across the muscle (Marcotte, West, & Baar, 2015), to which the first molecular regulator of load-induced skeletal muscle hypertrophy was identified as the mammalian target of rapamycin (mTOR) (Baar & Esser, 1999). In this piece of seminal research, Baar and Esser (1999) highlighted that there is a load-dependent increase in mTOR activity after resistance exercise that correlates with the resulting increase in muscle mass following training. In addition to mechanical loading, mTOR may be independently stimulated by growth factors and amino acids (Marcotte et al., 2015). The final step in the mTOR pathway is shared by stimulation from both mechanical load and growth factors which is why there is very little additive effect. In comparison, amino acids signal in parallel, allowing increased mTOR signalling when amino acids are supplied together with either growth factors or mechanical loading (Marcotte et al., 2015).

Building on the early work by Baar and Esser (1999), more recent evidence suggests that load does not determine the increase in muscle size that occurs with training. Here, the most important aspect for muscle hypertrophy is momentary muscular failure whereby low loads lifted to positive failure induce similar gains in muscle mass compared to using a high load and fewer repetitions to reach failure (Mitchell et al., 2012; Schoenfeld, Peterson, Ogborn, Contreras, & Sonmez, 2015). The proposed mechanism is that at failure, all motor units are recruited, regardless of load (Counts et al., 2016). However, pertinent to this discussion is that

despite similar increases in muscle size with both low and high loads, high loads are required to maximise strength gains (Schoenfeld, Peterson, et al., 2015).

Continual development progresses understanding in the field and recent research has highlighted other areas that may modulate adaptations to resistance training including ribosome biogenesis (Wen, Alimov, & McCarthy, 2016) and myostatin signalling (Hughes et al., 2018). However, further research is required before their role is fully understood. It is important to recognise the complex interaction between several components of the post-exercise response and that their roles may not be independent of one another. A mechanistic understanding of the determinants of strength and the array of adaptations to resistance exercise will aid in the design of training programmes with the goal of enhancing skeletal muscle size and strength.

2.4.4 Manipulating Exercise-Induced Hormesis

The dichotomy of acute physiological responses is related to the question of whether the goal is to accelerate recovery or to promote adaptation. Investigators have therefore attempted to optimise recovery and/or adaptation processes by manipulating the extent of these responses within the concept of hormesis (Figure 2.2).

Strategies that may dampen the exercise response include: non-steroidal anti-inflammatory drugs (NSAIDs), antioxidant supplements and cryotherapy (Peake, Markworth, et al., 2015) (Figure 2.2). These strategies are purported to reduce symptoms of inflammation and/or oxidative stress to counter the associated deleterious consequences and accelerate recovery following exercise (Barnett, 2006; Bryer & Goldfarb, 2006; Leeder, Gissane, van Someren, Gregson, & Howatson, 2011). However, recent reports suggest that the chronic use of these strategies may blunt long-term training adaptations (Michailidis et al., 2013; Roberts et al., 2015; Trappe et al., 2002). Findings such as these provide further support for the role of acute physiological responses in promoting beneficial training adaptations.

On the opposing side of the hormesis spectrum, several strategies may also enhance the exercise stimulus including: training with low muscle glycogen content, blood-flow restricted exercise and the application of heat to muscle (Peake, Markworth, et al., 2015) (Figure 2.2). The goal of these strategies is to manipulate the acute physiological responses in relation to an exercise session to promote greater adaptation. Although this area is in its infancy, several reports have shown the successful application of this concept to: enhance mitochondrial-related cell signalling following aerobic exercise (Impey et al., 2016), and increase strength and hypertrophy using low intensity resistance training when the training is performed with blood-flow restriction (Loenneke, Wilson, Marín, Zourdos, & Bemben, 2012) or combined with heating (Goto, 2007).

For practitioners that are interested in both accelerating recovery following competition, as well as promoting adaptation to training, there is clearly relevance for the application of different strategies. Optimising acute physiological responses depends on the context of the session within the recovery/adaptation paradigm. Research that can provide insights into the impact of a given strategy on acute physiological responses and then how this affects recovery and adaptation will develop pertinent recommendations regarding its application in the real-world. One such strategy that has relevance to the recovery adaptation paradigm is hot water immersion and will be discussed in the following section.

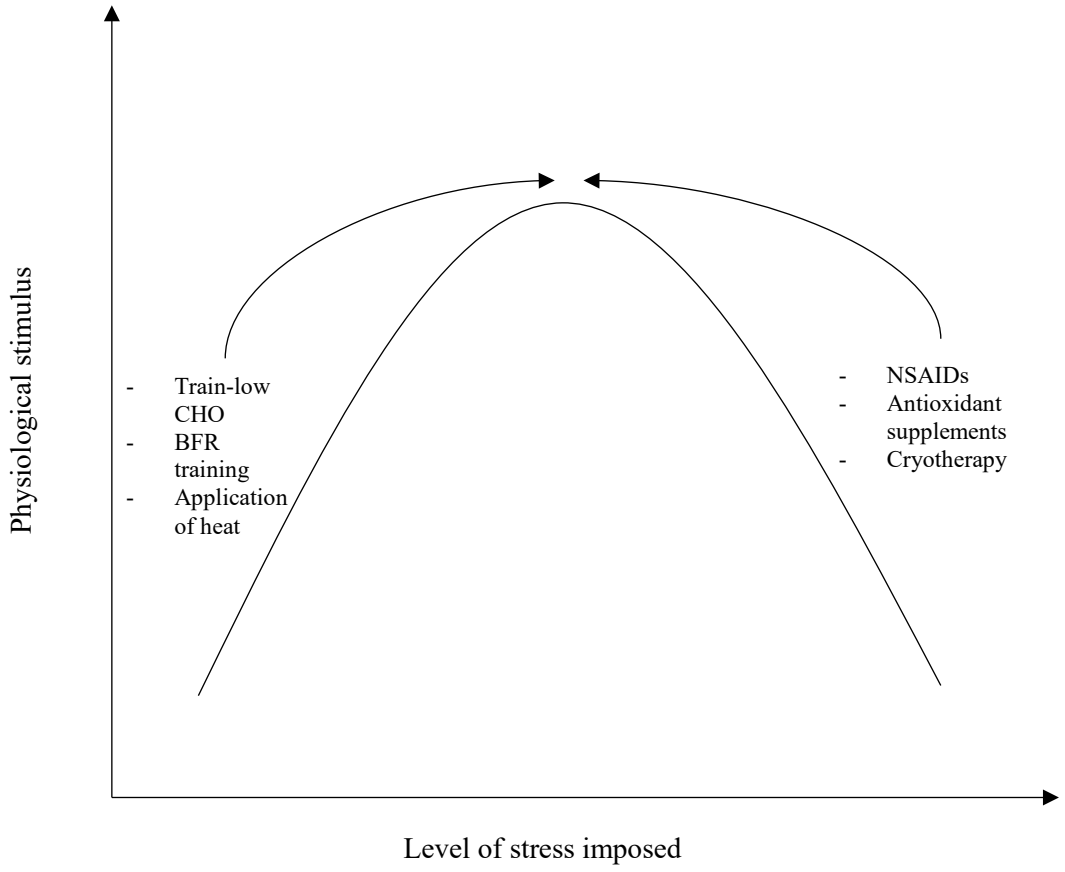

Figure 2.2 Typical hormesis curve and strategies to either enhance/dampen exercise-induced hormesis. CHO, carbohydrate; BFR, blood-flow restriction; NSAIDs, non-steroidal anti-inflammatory drugs.

2.5 Hot Water Immersion

2.5.1 Introduction

The term kaumatherapy, derived from the Greek 'kauma' meaning heat, encompasses methods to increase body temperature and is the opposite of the popular cryotherapy treatments (in Greek 'cryo' meaning cold) (Méline, Watier, & Sanchez, 2017). Kaumatherapy techniques include heat pads, microwave therapy, heat chambers, and hot water immersion (HWI) consisting of the submersion of parts of the body in hot water. Superficial application of heat has been typically used in physiotherapy and aside from basic physiological responses,

the effects on performance, recovery and adaptation remain poorly understood (Méline et al., 2017; Vaile et al., 2010; Wilcock et al., 2006). Within the scope of this thesis, the following sections will focus on HWI, however other methods of kaumatherapy will be referred to when appropriate.

2.5.2 Physiological Responses

The primary effects of kaumatherapy are brought about by increases in subcutaneous and cutaneous tissue temperature which induces peripheral vasodilation and a subsequent increase in blood flow (Wilcock et al., 2006) (Figure 2.3). The effect of kaumatherapy on intramuscular temperature and blood flow will be discussed in the following sections.

2.5.2.1 Intramuscular Temperature

Hot water immersion has been shown to induce increases in intramuscular temperature (3.6°C) at a depth of 3 cm, similar to that of moderate aerobic exercise, following a 60 minute heating protocol at 45°C (Morton et al., 2007). In contrast, others have reported little effect on tissue temperature at a depth of 1 cm when a hot-pack was applied for 5 minutes as part of a contrast therapy (Myrer, Measom, Durrant, & Fellingham, 1997). Other reports have shown intramuscular temperature at 1 cm to be elevated 1.1°C following 5 minutes of hot water immersion at 40°C (Myrer, Draper, & Durrant, 1994), highlighting variability in the potency of different heating methods. The temperature gradient of skeletal muscle dictates that shallow depths will be more readily affected by superficial temperature changes (Faulkner et al., 2012), suggesting the increase in intramuscular temperature at shallow depths would have been even more pronounced in the study by Morton et al. (2007). In addition to the temperature gradient, several other factors will also impact intramuscular temperature with heat therapy including: the surface area of heat application, duration of exposure, environmental conditions and body composition (Myrer et al., 1994). It was originally proposed that reaching intramuscular temperatures of 40°C was required to produce significant physiological responses (Lehmann, Warren, & Scham, 1974). However, several reports have shown changes in physiological responses with heat therapy protocols that would have been unlikely to elevate intramuscular temperature to 40°C (Goto, 2007; Touchberry et al., 2012; Vaile et al., 2008b; Viitasalo et al., 1995). Therefore, a consensus has not been achieved on the level of intramuscular temperature required to induce physiological responses or details of the heat therapy protocol to support this. For athletic populations interested in applying heat therapy following resistance exercise, it would be of benefit to understand the degree of change in intramuscular temperature and the associated physiological effects with ecologically valid protocols.

2.5.2.2 Blood Flow

A rise in superficial tissue temperature initially causes an increase in cutaneous blood flow, due to peripheral vasodilation (Bonde-Petersen, Schultz-Pedersen, & Dragsted, 1992; Knight & Londeree, 1979) (Figure 2.3). Total peripheral resistance may be seen to reduce as a characteristic of water immersion *per se*, although a greater reduction is seen with increasing immersion temperatures (Weston, O'hare, Evans, & Corrall, 1987). Concomitant to a reduced peripheral resistance is a fall in diastolic blood pressure and a progressive rise in cardiac output, which shows a ~120% increase compared with baseline values, attributable to both a rise in stroke volume and tachycardia (Weston et al., 1987). Together, the increased cardiac output and a lower peripheral resistance allows an increase in cutaneous and subcutaneous blood flow (Wilcock et al., 2006).

Such an increase in tissue blood flow may facilitate increases in metabolism, nutrient delivery and waste removal through an increased permeability of cellular, lymphatic and capillary vessels (Wilcock et al., 2006) (Figure 2.3). Supporting evidence for this theory is shown as warm underwater jet-massage was seen to increase the release of intramuscular proteins (e.g. CK and myoglobin) into the blood during an intensified strength/power training period (Viitasalo et al., 1995). When exercise intensity is such that muscle cell membrane integrity is compromised, intramuscular proteins such as CK leak into the interstitial fluid before being taken up by the lymphatic system and returned into the circulation (Bijsterbosch et al., 1985). The rise in CK with warm underwater jet-massage compared to a control condition suggests that temperature-induced increases in tissue blood flow may enable an accelerated removal from the extracellular space (Viitasalo et al., 1995). Others have not reported increased circulating intramuscular proteins following post-exercise hot water immersion (Pournot et al., 2011; Vaile et al., 2008b), and therefore further research is required to elucidate the effects of kaumatherapy on circulating markers of acute physiological responses.

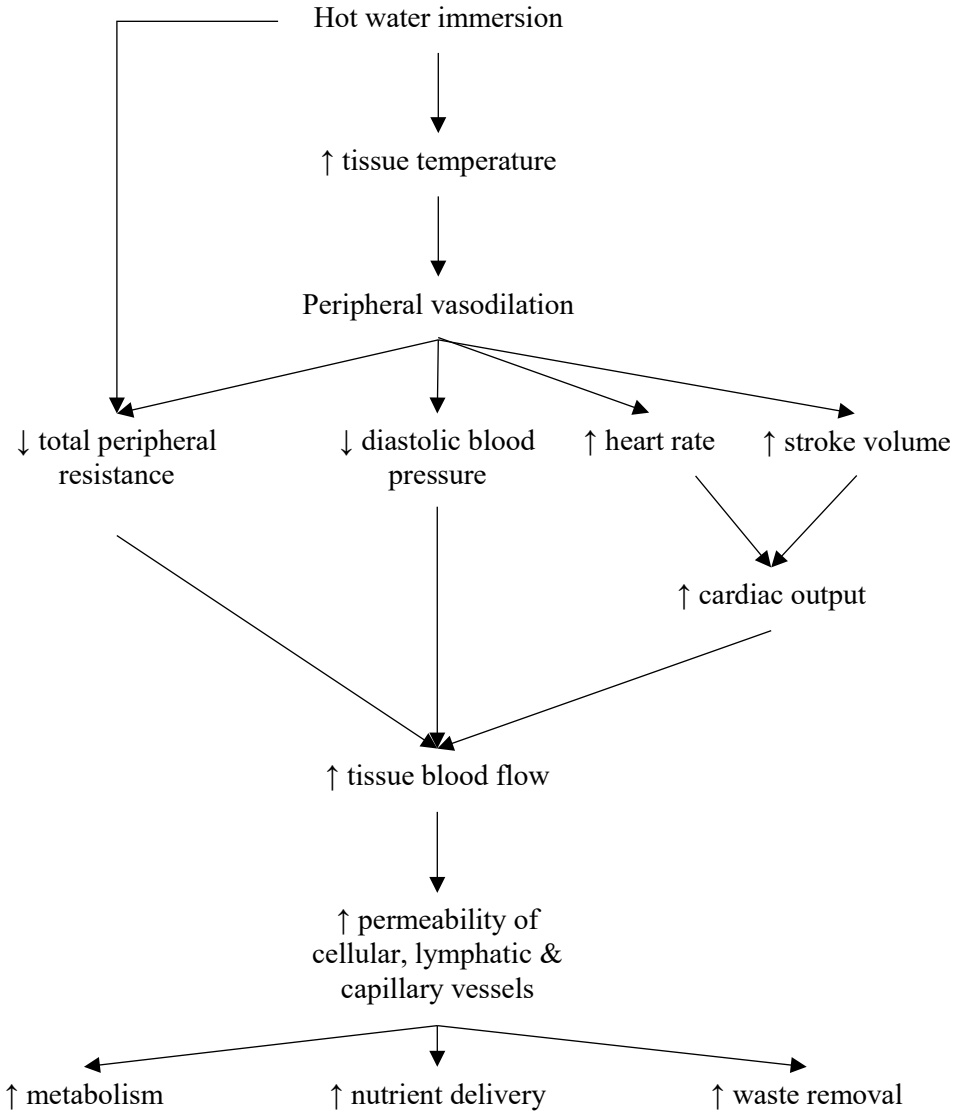

Figure 2.3 Overview of the proposed physiological responses to hot water immersion.

2.5.3 Recovery

In contrast to the much-publicised research into cryotherapy, relatively little evidence exists to detail the effects of kaumatherapy, especially with regards to recovery from and adaptation to exercise. This is despite anecdotal reports of its use with athletic populations (Vaile et al., 2010), and the modern advent of saunas, steam rooms and immersion pools in an increasing number of leisure facilities, which make kaumatherapy an easily accessible strategy to recreational exercisers. Therefore, an appreciation of its effects is warranted to better inform recommendations to a variety of populations.

Following an eccentric exercise protocol, 14 minutes of HWI at 38°C enhanced the recovery of muscle function at 24, 48 and 72 h post-exercise compared to a passive recovery control condition (Vaile et al., 2008b). Viitasalo et al. (1995) also reported beneficial effects of a warm underwater jet-massage protocol which resulted in a smaller decline in jumping power and a smaller increase in continuous jumping contact time compared to no treatment during a strength/power intensified training week. Superficial heat application has been proposed to increase neural transmission (Cotts, Knight, Myrer, & Schulthies, 2004) which may explain in part the beneficial effects of kaumatherapy on return of muscle function following exercise, although further research is clearly warranted as others have shown no such effects (Kuligowski et al., 1998; Pournot et al., 2011; Vaile et al., 2008a). Differences in water temperature, exercise modality and affected limbs may explain some of the discrepancies in these findings whereby temperatures ≥38°C and eccentric exercise targeting the lower limbs show more promising results.

Research into chronic pain has suggested that heat application may be beneficial in reducing feelings of pain when applied continuously (8 hours/day) over a sustained period (2-5 days) (Nadler, Steiner, Erasala, et al., 2003; Nadler et al., 2002; Nadler, Steiner, Petty, et al., 2003). In an exercise setting, only one report suggests that the application of superficial heat reduces the rise in muscle soreness after exercise (Viitasalo et al., 1995), with the majority of research weighted in favour of no effect of kaumatherapy (Kuligowski et al., 1998; Pournot et al., 2011; Vaile et al., 2008b). The proposed mechanism by which topical heat application may reduce pain is through the gate control theory (Melzack & Wall, 1967). The gate control theory suggests that inhibition of nociception in the dorsal horn of the spinal cord prevents feelings of pain, and since warming the skin increases mechanoreceptor sensitivity, heat application may interfere with nociception (Nadler, Steiner, Petty, et al., 2003). However, research into the short-term application of heat after exercise is equivocal with regards to its effect on muscle soreness and pain warranting further investigation (Wilcock et al., 2006).

Superficial heat application may increase oedema and swelling following joint injury, suggesting a role of heat in inducing the inflammatory response (Coté, Prentice Jr, Hooker, & Shields, 1988). Only two studies have collected inflammatory markers from the blood after exercise, both of which have shown no effect of kaumatherapy (Pournot et al., 2011; Vaile et al., 2008b). Clearly there are differences in both the method of assessing inflammation (swelling versus blood markers) and the method to induce the inflammatory response (joint injury versus exercise) which make direct comparisons difficult. Given the lack of research in an exercise setting, it would be prudent for future investigations to assess the impact of kaumatherapy on the inflammatory response using sensitive markers.

Current understanding suggests that HWI may manipulate acute physiological responses that could accelerate recovery from resistance exercise. Equivocal findings make definitive conclusions difficult at this stage and may be attributed to variations in heat therapy protocols that have been employed, differences in exercise modality and the measures of acute physiological responses that have been used to provide these conclusions. Drawing on this literature, it may be possible to inform future study design to offer the most promise in this area, whilst consideration should also be made to enhance the ecological validity of these findings so that they can be applied in the real world.

2.5.4 Adaptation

It is perhaps unsurprising that given the paucity of literature to demonstrate the effects of kaumatherapy on acute physiological responses, that research to investigate the long-term effects on exercise adaptation is scarce, to which there has been a call to arms from recent authors (Méline et al., 2017). This comes after post-exercise whole body heat stress was seen to additively enhance endurance training-induced mitochondrial adaptations in mouse skeletal muscle (Tamura et al., 2014), gaining attention for heat stress to be a stimulus for promoting cellular and systemic adaptation of the endurance phenotype (Hawley, Lundby, Cotter, & Burke, 2018).

Emerging evidence also suggests that kaumatherapy may promote beneficial adaptations related to resistance exercise. Heat stress has been shown to upregulate key anabolic signalling pathways and protein expression both independently (Kobayashi et al., 2005; Uehara et al., 2004; Yoshihara et al., 2013) and following a mechanical stimulus (Goto et al., 2003) in animals. Heat stress may also provide a protective role against muscle disuse atrophy induced by hind limb unweighting in rats (Naito et al., 2000).

Perhaps the most compelling evidence in favour of kaumatherapy was found by Kakigi et al. (2011). This study demonstrated for the first time in human skeletal muscle that heat stress induced by a microwave therapy unit in combination with a bout of resistance exercise enhanced the phosphorylation of molecules within the mTOR pathway compared to resistance exercise in isolation. Although speculative as it was not measured, the authors suggested that a rise in both intramuscular temperature and tissue blood flow contributed to a higher phosphorylation level. Previous research has reported that increasing blood flow enhances muscle protein synthesis as well as Akt and mTOR phosphorylation (Fujita et al., 2007). It was concluded that resistance exercise with the addition of heat stress may be a useful tool to promote muscle mass (Kakigi et al., 2011).

In line with the upregulation of anabolic processes, heat stress enhanced both isometric force and cross-sectional area of the elbow flexors following 10 weeks of resistance training (Goto,

2007). It is noteworthy that this study utilised a within-subject design whereby the non-dominant limb received heat stress in addition to the resistance exercise, whilst the dominant limb performed resistance exercise alone. Previous research has demonstrated that both exercise (Hendy & Lamon, 2017) and temperature (Allan et al., 2017) may exert systemic effects via cross-talk which may potentially confound the results of this study. Congruent with this suggestion is the findings of others that showed neither a positive nor negative effect of during and post-exercise muscle heating on hypertrophy and strength with resistance training (Stadnyk, Rehrer, Handcock, Meredith-Jones, & Cotter, 2018). Despite this, other reports have shown passive heat exposure to improve skeletal muscle contractile function following an 11 day acclimation period (Racinais, Wilson, & Périard, 2016).

Promising evidence exists to suggest that kaumatherapy may promote beneficial adaptations in addition to resistance exercise in human skeletal muscle, although further research is required to determine the potential of this strategy to enhance adaptations for individuals engaged in resistance training (McGorm, Roberts, Coombes, & Peake, 2018). Given the evidence to suggest HWI may influence acute physiological responses, it would be of interest to investigate how manipulation of these acute physiological responses may impact training adaptation. This would enhance our understanding of both the effects of HWI on recovery and adaptation as well as provide insights into the influence of acute physiological responses on long-term training adaptation.

2.5.5 Methodological Considerations

As highlighted previously, there are several methods related to kaumatherapy which can exert a superficial heat stimulus to the body. For the purposes of this literature review, the following section will focus on methodological considerations related to kaumatherapy via hot water immersion.

The primary goal of applying hot water immersion is typically to increase tissue temperature, however a range of protocols have been employed suggesting that there is not a current consensus on an optimal strategy (Kuligowski et al., 1998; Morton et al., 2007; Pournot et al., 2011; Vaile et al., 2008a, 2008b; Viitasalo et al., 1995). Only one study has measured intramuscular temperature in response to HWI and showed increases of 3.6°C (35.9 to 39.5°C) following 1 h of single limb immersion at 45°C (Morton et al., 2007). Extrapolating data from other methods of kaumatherapy reveals tissue temperatures of 38°C result in beneficial adaptations to muscle size and strength following a resistance training programme (Goto, 2007). Participants in the study by Morton et al. (2007) had reached an intramuscular temperature of 38°C following 20 minutes of immersion, which was coincidentally the first point of measurement following the commencement of the protocol. It's therefore not fully

known if shorter durations of HWI may be able to raise intramuscular temperature to physiologically relevant levels. Other considerations for application of HWI in the real world include ecological validity and subjective comfort. It is unlikely that elite athletes would have the time to perform immersions with a prolonged duration, such that in CWI research the typical recommended duration is 10-15 mins (Leeder et al., 2011). Water temperatures greater than 40°C may be uncomfortable due to the heat being applied to the skin (McGorm et al., 2018) and participants may report feelings of dizziness (Hooper, 1999). Modern leisure facilities enhance the availability of HWI via Jacuzzi's, which are in the region of ~40°C (Hooper, 1999). Together it seems appropriate for future research to utilise protocols of ~40°C for a duration of 10-15 mins which would enhance the applicability of the findings for use in the real world.

A consideration specific to water immersion varieties of kaumatherapy is that of hydrostatic pressure (Vaile et al., 2008a). During water immersion, a pressure is exerted on the body which causes an inward and upward displacement of fluid from the extremities, increasing central blood volume (Arborelius, Ballidin, Lilja, & Lundgren, 1972; Löllgen, v. Nieding, Koppenhagen, Kersting, & Just, 1981; Wilcock et al., 2006). As one of the goals of hot water immersion is to increase tissue blood flow, the effect of hydrostatic pressure within previous research may have counteracted some of this process. To neutralise a pressure increase of 0.74 mmHg for every 1 cm increase in water depth (Wilcock et al., 2006), Leeder et al. (2015) employed a seated immersion group (hydrostatic pressure at ankle approximately 40 mmHg) as a comparator to a standing immersion group (hydrostatic pressure at ankle approximately 111 mmHg). Hydrostatic pressure may therefore be a confounding variable within HWI research and future studies should control for its effects by using seated immersion protocols to optimise increases in tissue blood flow.

Despite a lack of consensus on an optimal protocol, calls for individualisation of HWI strategies may be required based upon differences in body composition (Stephens, Argus, & Driller, 2014). This study reported that following HWI (15 minutes at 38°C), a low-fat group had a higher core temperature 5 minutes post-immersion and a higher rating of thermal sensation at 15 minutes post-immersion compared to a high-fat group. Intramuscular temperature was not measured in this study, however deeper muscle tissue has previously been shown to take longer to cool and rewarm following cryotherapy (Myrer, Myrer, Measom, Fellingham, & Evers, 2001). Future research is required to provide further insights into the effect of body composition on changes in tissue temperature following HWI. In the absence of this information, it may be prudent for investigators to recruit homogenous cohorts and to match independent groups to control for differences in body composition when comparing the effects of HWI.

Several methodological considerations should be incorporated into future HWI research to draw upon the findings of previous research, to ensure appropriate control measures and to enhance future ecological validity of protocols.

2.6 Summary

Following resistance exercise there is a series of acute physiological responses, categorised into a primary and secondary phase, which may cause the appearance of intramuscular proteins in the blood, inflammation, and oxidative stress. As a result, muscle function could be impaired, muscle soreness may be present and disruptions to other aspects such as feelings of stress and sleep arise. Although this process is generally accepted, several questions exist with regards to appropriately characterising the magnitude and time course of this response which relate to the sensitivity of the measures which are used to do so. Information as to the most sensitive measures to characterise acute physiological responses would be of interest to practitioners who are managing an athletes training load/readiness to train as well as researchers who are looking to develop strategies to accelerate recovery from a bout of resistance exercise.

More recently, there is growing evidence that manipulating acute physiological responses post-exercise may influence training adaptation. Within the concept of hormesis, strategies may either dampen or enhance acute physiological responses with the goal of optimising the exercise stimulus. However, a dichotomy is evident between the contexts in which an athlete may need to prioritise recovery or adaptation. This becomes prevalent when recent reports suggest that certain strategies that aim to dampen the exercise response may inhibit training adaptations. In respect of the current thesis, post-exercise HWI is a strategy that has received limited attention. This is despite the theoretical mechanisms which have been proposed for how HWI may manipulate acute physiological responses to accelerate exercise recovery and/or enhance training adaptation. However, differences in HWI protocols, timing of application and exercise modality make conclusions problematic. This may arise from a lack of ecological validity within the research area which needs to be addressed if this strategy is to be employed in the real world. To date no research has investigated the chronic use of HWI alongside a resistance training programme. Given the importance of acute physiological responses in the recovery from and adaptation to resistance exercise, it would be of interest to understand the role of HWI in manipulating these.

This review has identified several areas that require further investigation and consequently some of these will be addressed in the subsequent experimental chapters by raising the following questions:

1. What are the most sensitive measures related to the acute physiological responses following resistance exercise? And using these measures, what is the magnitude and time course of this response?
2. Can HWI manipulate the acute physiological responses following resistance exercise? And how does this influence exercise recovery?
3. Does regular HWI influence adaptation to a resistance training programme? And what is the effect of HWI on acute physiological responses at the end of the training period?

3 General Methods

3.1 General Methods

This thesis consists of three progressive studies designed to investigate the effect of HWI on acute physiological responses and the subsequent impact on recovery and adaptation. Each investigation was undertaken following institutional ethical approval. This chapter describes common methodological procedures used through the course of studies and acts as a reference point for experimental chapters presented later in this thesis.

3.2 Participant Recruitment

Throughout the course of investigation, resistance-trained males between the ages of 18-35 years were recruited. Participants were considered to be resistance-trained if they had performed ≥3 resistance sessions per week for ≥2 years with a minimum of one session per week including exercises that targeted the lower limbs (Buckner et al., 2017). Participants were recruited via emails, social media and poster adverts aimed at the population described. After volunteering for a study, participants were provided with detailed instructions regarding the study requirements and were afforded the opportunity to meet with the principal investigator to ask any questions. Associated risks and benefits of involvement were discussed prior to gaining written informed consent (Appendix 1) and completing a health and training questionnaire (Appendix 2). Participants were fully familiarised with testing procedures on two separate occasions, prior to commencement of the experimental period, which are outlined in further detail within each experimental chapter.

3.3 Nutritional Control

To minimise the influence of dietary intake as a confounding factor, participants were instructed to maintain their habitual diet throughout each study and asked to record this in a food diary (Appendix 3). The specific use of the food diary for each study is described in more detail in the relevant experimental chapter.

3.4 Strength Assessments

3.4.1 6 repetition maximum

Strength assessments were performed using a 6 repetition maximum (RM) testing protocol in accordance with recognised guidelines (Haff & Triplett, 2015). Following a standardised warm-up, participants performed three sets of six repetitions of back squat with a progressively increasing load that corresponded to 50, 75 and 90% of their perceived 6 RM. Participants then performed sets of six repetitions with an increasing load for the determination of 6 RM with 2 min rest afforded between attempts. All 6 RM determinations were made within four attempts which were deemed successful by an investigator if a participant had reached a position in which the thigh was at least parallel to the floor. Participants repeated the above procedure for the determination of 6 RM on the front squat, good mornings and

Bulgarian split-squat exercises with successful attempts determined by an investigator against standardised techniques. During the Bulgarian split-squat, participants placed the top of the toes of the trail leg on a 30 cm platform (McCurdy et al., 2010). The lead leg was placed approximately 99-114 cm from the front edge of the platform supporting the trail leg. Participants were then required to squat to a depth where the thigh of the lead leg was parallel to the ground before returning to the start position. These exercises were chosen to target a range of lower limb musculature and are commonly included in strength and conditioning programmes (Haff & Triplett, 2015). All resistance exercises were performed using free weights and a standard 20 kg bar (ELEIKO SPORT, Illinois, USA).

3.4.2 1 repetition maximum

For studies 2 and 3, maximal lower-body strength was assessed by 1 RM testing in the back squat consistent with recognized guidelines established by the National Strength and Conditioning Association (Baechle & Earle, 2008). The back squat was chosen as it is a well-established measure of maximal strength (Schoenfeld et al., 2016). Following the standardised warm up, participants performed a set of 5 repetitions at a load that corresponded to approximately 50% of their perceived 1 RM, followed by two sets of 2-3 repetitions at a load which corresponded to ~60-80% of perceived 1 RM with 2 minutes rest afforded between sets. Participants then performed sets of 1 repetition of an increasing load for 1 RM determination with 3 minutes rest afforded between attempts. All 1 RM determinations were made within 5 attempts. For an attempt to be deemed successful by the investigator, participants were required to squat to a depth so that the top of the thigh was at least parallel to the ground.

3.5 Resistance Exercise Session

The same resistance exercise session was used in all studies across this thesis. Following the standardised warm up (Appendix 4), participants performed three sets of six repetitions of back squat with a load corresponding to 50, 75 and 90% 6 RM. Participants then performed four sets of six repetitions with a load corresponding to 6 RM for the following exercises: back squat, front squat, good mornings and Bulgarian split-squat. The intensity (100% 6 RM or ~85% 1 RM) and volume (12 sets targeting the quadriceps muscle group) of the session were selected based upon recommendations that loads of 80-95% 1 RM elicit maximal gains in strength (Peterson, Rhea, & Alvar, 2005), and hypertrophy (Fry, 2004). The performance of at least 8-10 weekly sets per muscle group has also been suggested to be required to maximise increases in muscle strength (Peterson et al., 2005) and size (Schoenfeld et al., 2016) in trained individuals. Participants were instructed to perform the eccentric phase of the exercises in a controlled fashion lasting approximately two seconds, whilst the concentric phase was to be performed with maximal acceleration. This method of lifting was chosen given the suggestion that it is the intended rather than the actual velocity that determines the velocity-specific

training response (Behm & Sale, 1993). Two minutes rest was afforded between sets and exercises, which has been recommended as a minimum for maximising gains in muscle size (Schoenfeld, Pope, et al., 2015). If a participant was unable to perform six repetitions of the prescribed load with correct technique (as determined by an investigator), the load was reduced such that six repetitions could be maintained per set. Pilot testing demonstrated that this protocol resulted in changes in acute physiological response measures (i.e. increased CK and muscle soreness) (Appendix 5).

3.6 Interventions

The HWI or passive recovery (PAS) interventions used in study 2 and 3 were performed within 10 min post-completion of the resistance exercise session. Participants in the HWI group sat in an inflatable bath (iSprint, iCoolsport, Miami, Australia) and were required to submerge their legs in the water up to their waist in a seated position (hip angle of ~90°), with their legs outstretched and relaxed. Water temperature was maintained at 40°C using a circulatory heating unit (iCool dual temperature LITE, iCoolsport, Miami, Australia) and participants were required to remain immersed for 10 min. Participants in the PAS group sat on a physiotherapy bed and were required to remain still in a seated position (hip angle of ~90°), with their legs outstretched and relaxed for 10 min. To avoid any external confounding influences on temperature, participants were required to remain in the laboratory until after all assessments were complete for that visit.

3.7 Body Composition

Body composition was assessed in study 2 and 3 by dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy, GE Healthcare, Bucks, UK). Participants were required to perform the scan following a minimum of a three hour fast and with all metal artefacts removed. Body mass was measured using a digital column scale (seca 703, seca Ltd., Hamburg, Germany). In addition to regular machine calibration, a standard quality assurance procedure was passed prior to each scan (Bell, Furber, van Someren, Antón-Solanas, & Swart, 2016). Participants then underwent a 'total body' scan, which required maintenance of a still supine position for the duration of the scan. The scan was performed in accordance with the manufacturer's guidelines for patient positioning and was performed by an investigator fully trained in the procedure. The scan was analysed using encore software, version 14.10 (GE Healthcare).

3.8 Muscle Function

3.8.1 Maximal voluntary isometric contraction

Participants were seated on the dynamometer chair (study 1, Humac Norm, CSMi, Massachusetts, USA; study 2 and 3, Biodex 3, Biodex Medical Systems, NY, USA) with a hip joint angle of 90° and a knee joint angle of 70° (Eddens et al., 2017), set by the investigator

using a goniometer. A knee joint angle of 70° has been shown to be sensitive to detect reduced muscle function following eccentric exercise, with no difference between this angle and the torque produced at 90° (McHugh & Tetro, 2003). Participants completed a standardised warm-up consisting of efforts at 50, 75 and 90% of perceived maximal force. Participants then performed three MVICs of the right limb, each lasting 3 sec, with standardised verbal instruction and encouragement provided throughout. Sixty seconds rest was afforded between attempts with peak force (N) recorded and the best attempt used for subsequent analysis.

3.8.2 Isometric squat

For study 2 and 3, participants were instructed to stand on a Kistler force plate (portable multi-component force plate type 9286BA, Kistler, Winterthur, Switzerland) in a custom designed rack (Absolute Performance, Cardiff, UK). The bar was positioned to be in line with the participant's sternum, with the height recorded and maintained for subsequent trials. Participants were asked to rest the bar on the upper trapezius muscle and complete three warm-up efforts at 50, 75 and 90% of perceived maximal force before completing three good attempts where they were instructed to contract "as hard and as fast as possible" (Bishop, Turner, Jarvis, Chavda, & Read, 2017). Prior to each attempt, the participant prepared themselves in the ready position and motionless on the force plate for at least 1 second to obtain bodyweight and the baseline period. Initiation of the contraction was identified as the first force value greater than 5 standard deviations (SD) of the baseline period (Chavda et al., 2018). Trials were rejected if there was any evidence of pre-tension or visible countermovement. Each attempt lasted approximately 5 seconds with peak force and RFD calculated from the best attempt and used for subsequent analysis. Peak force refers to the greatest force value produced minus the bodyweight calculated from the baseline period. Rate of force development was calculated as the change in force ($N \cdot s^{-1}$) following the initiation of the contraction in the time intervals 0-100 ms (RFD_{0-100}) and 100-200 ms ($RFD_{100-200}$). These RFD time intervals have been suggested to differentiate the mechanisms of force loss following eccentric exercise, representing neuromuscular function and muscle damage, respectively, when assessing isometric actions (Jenkins et al., 2014; Peñailillo et al., 2015; Tillin et al., 2012).

3.9 Active Muscle Soreness

Active muscle soreness was determined using a 200 mm visual analogue scale (VAS) with "no pain" indicated at one end and "pain/soreness as bad as it could be" at the other (Appendix 6) (Bell, Walshe, Davison, Stevenson, & Howatson, 2014). Participants were instructed to stand with hands on hips and feet shoulder width apart prior to performing a squat to a depth whereby the thigh was parallel to the floor. Upon completion, participants indicated the pain

felt in the lower limbs by drawing a line on the VAS, which was converted to a percentage of the total line for subsequent analysis (Hopkins, 2013).

3.10 Blood Sample Collection and Analysis

Venous blood samples were collected using the venepuncture technique from a vein in the ante-cubital fossa region by a trained phlebotomist in all three studies. Blood was collected into two 5 mL serum separator tubes and left to clot for; 5 min prior to being centrifuged at 4000 *g*, 23°C for 3 min (study 1), and 30-60 min prior to being centrifuged at 3000 *g*, 23°C for 8 min (study 2 and 3). Participants in study 1 also had blood collected into two 2.5 mL di-potassium ethylene diamine tetra-acetic acid (EDTA) tubes which were left to clot for 5 min prior to being centrifuged at 2300 *g*, 23°C for 10 min. The serum/plasma was then removed and immediately stored in aliquots at -80°C for later analysis.

In addition to the following analyses, other measures were included in study 1 which will be referred to in more detail in the relevant experimental chapter.

3.10.1 Creatine Kinase Analysis

For study 1, serum CK was determined using a CK NAC-activated kit (Randox Laboratories Ltd, County Antrim, UK). Ten µL of sample was mixed with 500 µL reagent and absorbance was read at 37°C on an Rx Monza clinical chemistry analyser (Randox Laboratories Ltd, County Antrim, UK). For study 2 and 3, serum CK was determined by electrochemiluminescence using an automated analyser (Roche c702 chemistry module, Roche Diagnostics Ltd., UK). The intra- and inter-assay coefficient of variation (CV) were 2.3% and 3.9% for study 1, and 0.5% and 1.4% for study 2 and 3, respectively.

3.10.2 High Sensitivity C-Reactive Protein Analysis

For study 1, serum high sensitivity C-reactive protein (hsCRP) was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems, Inc., Minnesota, USA). For study 2 and 3, serum hsCRP was determined by electrochemiluminescence using an automated analyser (Roche c702 chemistry module, Roche Diagnostics Ltd., UK). The intra- and inter-assay CV were 3.8-8.3% and 6.0-7.0% for study 1, and 1.0% and 5.3% for study 2 and 3, respectively.

3.10.3 Interleukin-6 and Interleukin-10 Analysis

Throughout the course of investigation, serum IL-6 and IL-10 were determined by an ELISA using commercially available kits (Invitrogen Corporation, California, USA). The intra- and inter-assay CV were 5.1-7.7% and 6.5-9.3% (IL-6, study 1), 6.2% and 7.9% (IL-6, study 2 and 3), and <10% and <10% (IL-10, study 1, 2 and 3), respectively.

3.10.4 Matrix Metalloproteinase-9 Analysis

Throughout the course of investigation, serum MMP-9 was determined by an ELISA using a commercially available kit (Invitrogen Corporation, California, USA). The intra- and inter-assay CV was 3.3-4.6% and 6.9-8.0% for study 1, and 7.3% and 10% for study 2 and 3, respectively.

3.11 Statistical Analysis

Throughout the course of investigation, data were analysed by making probabilistic magnitude-based inferences about the observed magnitude of the effect to assess the likelihood that changes are real using the methods described by Batterham and Hopkins (2006). Methods of statistical analysis is currently a contentious issue within the field of sport and exercise science. The recent trend to move away from the traditional null-hypothesis testing which focuses on an arbitrary *p* value in an absolute effect versus non-effect interpretation (Rowlands et al., 2007) has received rebuttal (Sainani, 2018). While the debate rolls on (Hopkins & Batterham, 2018), with no apparent sign of a conclusion approaching, applied physiologists are required to make a call on which method may best suit their investigation. Magnitude-based inferences are suggested to deal with the real world significance of outcomes and account for both the likelihood of an effect as well as its magnitude (Batterham & Hopkins, 2006), which may be relevant for practitioners. This method incorporates the smallest worthwhile change which allows probabilistic inferences to describe the effect, therefore increasing the accessibility to practitioners, support staff and athletes (Pyne et al., 2004; Rowlands et al., 2007). The selection of this statistical method therefore suits the applied nature of this thesis. Additionally, this method allows greater transparency of the data with the incorporation of effect sizes and confidence intervals that facilitate the reader to draw their own interpretations.

The smallest worthwhile change was standardised as a fraction of the between-subject SD at baseline from both the control and exercise weeks (study 1), and both groups (study 2 and 3) using the smallest change in the mean of 0.2 (Batterham & Hopkins, 2006). The smallest worthwhile change was used to determine the effect of the independent variable on each dependent variable using a spreadsheet designed for analysis of a crossover trial (study 1) (Hopkins, 2006), and analysis of a parallel groups trial (study 2 and 3) (Hopkins, 2006). Mean values of log-transformed data were back-transformed to provide mean percentage change and 90% confidence intervals, with measures that had large percentage changes presented as factors (Hopkins, 2003).

Standardised changes of 0.20, 0.60, 1.20, 2.0, and 4.0 were thresholds for small, moderate, large, very large and extremely large effects, respectively (Hopkins, Marshall, Batterham, &

Hanin, 2009). When the confidence interval for a change included both small positive and negative effects, the change was deemed unclear. For clear effects, the qualitative probabilities that the true effect was substantial was defined by the following scale: 25-75% possibly, 75-95% likely, 95-99.5% very likely, >99.5% most likely (Hopkins, 2006). Examples of different interpretations from magnitude-based inferences have been described elsewhere (Buchheit, 2016). Where presented, baseline values are reported as the absolute mean ± SD with all other values reported as the mean effect between HWI and PAS groups; ± or x/÷ 90% confidence intervals.

Where further analysis has been performed related to a specific study, this has been described in more detail in the relevant experimental chapter.

4 Assessing the Usefulness of Acute Physiological Responses Following Resistance Exercise: Sensitivity, Magnitude of Change and Time Course of Measures

This chapter assessed the usefulness of a variety of measures that are used to detail acute physiological responses following resistance exercise. The study utilised a crossover design, assessed measures through a relevant timescale (i.e. 2 h – 96 h post-exercise), recruited trained participants and employed a real-world exercise modality to enhance the ecological validity of the findings. The results suggest that several measures were able to demonstrate clear effects following resistance exercise. Additionally, we were able to provide a profile relating to the magnitude of change and time course for these measures with optimal sampling points identified which informed the acute physiological response measures used in subsequent chapters.

4.1 Introduction

Resistance training results in beneficial adaptations such as increased skeletal muscle size (Fry, 2004; Schoenfeld et al., 2016) and strength (Kraemer et al., 2002; Peterson et al., 2004), due in part to acute physiological responses (Peake, Markworth, et al., 2015; Schoenfeld, 2012). It is widely accepted that key aspects of the acute physiological response (hours and days post-exercise) are a transient reduction in muscle function, increased muscle soreness, increased swelling of the exercised musculature and increases in the appearance of markers of inflammatory processes and oxidative stress as well as intramuscular proteins and enzymes in the blood (Howatson & Van Someren, 2008).

Given the deleterious effects of resistance exercise on proximate performance, there has been keen interest to develop strategies to reduce aspects of the acute physiological response in order to enhance recovery processes (Peake, Neubauer, Della Gatta, & Nosaka, 2017). Conversely, recent reports have suggested that increasing the volume of these physiological responses may be desirable for those looking to enhance adaptive responses to exercise (Peake, Markworth, et al., 2015). To assess the true impact of these strategies, researchers need to know the usefulness of measures in order to detect meaningful change and produce suitable conclusions (Buchheit, Simpson, Al Haddad, Bourdon, & Mendez-Villanueva, 2012).

The usefulness of a measure is typically underpinned by: the typical error of the measurement (reliability), the magnitude of the change post-exercise compared with the typical error (signal:noise), and the smallest change in the measure that is of importance to researchers and practitioners, otherwise known as the smallest worthwhile change (Pyne et al., 2004). Previous research has demonstrated good reliability for a range of indirect measures (Howatson & Milak, 2009; Morton et al., 2005; Nunan, Howatson, & Van Someren, 2010; Pyne, 2003). However, only one of these studies (Morton et al., 2005) assessed reliability across a timescale that is reflective of that in which the measure is to be used e.g. to characterise the physiological response in the hours and days post-exercise (Atkinson & Nevill, 1998). While what is understood to be the classical physiological response to resistance exercise has been reviewed elsewhere (Clarkson & Hubal, 2002; Howatson & Van Someren, 2008), in order to be confident that these changes are real and not just due to random error, an understanding of the measures usefulness is important. Without this information, the documented changes in physiological response measures may simply be noise associated with within-subject variation. To appropriately characterise a measure's usefulness to detect changes following exercise requires a control condition identical to the experimental condition but void of the exercise stimulus as well as using the same population which was used to quantify reliability of the measures. This information would enable researchers and practitioners to use the most sensitive measures that would show real effects of strategies that aim to manipulate acute

physiological responses for the purposes of recovery and/or adaptation in order to deliver worthwhile impact.

The aim of the present study was twofold: (i) to investigate the sensitivity of a variety of indirect measures used to quantify the acute physiological response to resistance exercise in a trained cohort, and (ii) to provide a profile to characterise the magnitude of change and time course of this response. Assessments were scheduled to take place across a timescale that captures the profile of post-exercise physiological perturbations among individuals accustomed to resistance exercise to enhance the applicability of the results.

4.2 Methods

4.2.1 Participants

Eight resistance-trained males (age 21 ± 3 years, height 1.81 ± 0.08 m, body mass 82.0 ± 8.0 kg, back squat 6 repetition maximum [RM] 1.2 ± 0.2 x body mass) volunteered to take part in the study. The study was granted ethical approval (application number: 439) by the London Sports Institute Ethics Sub-Committee at Middlesex University.

4.2.2 Experimental Design

In a within-subject design, participants initially performed experimental sessions during a 'control week', which was void of an exercise stimulus in order to assess the reliability of a variety of measures across a relevant timescale. The following week, termed the 'exercise week', participants repeated this sequence of experimental sessions at the same time of day with the exception that they performed a bout of resistance exercise following baseline assessments. The exercise week enabled the characterisation of changes in the same measures from the control week following an acute bout of resistance exercise. Participants attended the laboratory on 12 separate occasions (Figure 4.1). During visit 1, participants were familiarised with all testing procedures as well as the resistance techniques used for the exercise session (back squat, front squat, good mornings, Bulgarian split-squat). Visit 2 consisted of a second familiarisation session in combination with a 6 RM strength assessment. During visit 3, which occurred 96 h following visit 2, participants completed baseline assessments prior to a 'control period' which consisted of 1 h rest. Participants then repeated the baseline assessments at 2 h, 6 h, 24 h (visit 4), 48 h (visit 5), 72 h (visit 6) and 96 h (visit 7) post the control period. Visit 8 took place 7 days following visit 3 and required participants to repeat the same testing procedures with the addition of a resistance exercise session that took place immediately after the baseline assessments. Visits 9-12 replicated visits 4-7 for post-exercise assessments. Prior to commencing exercise, participants completed a 5 min warm-up on a stationary cycle ergometer at a self-selected intensity (Wattbike, Nottingham, UK).

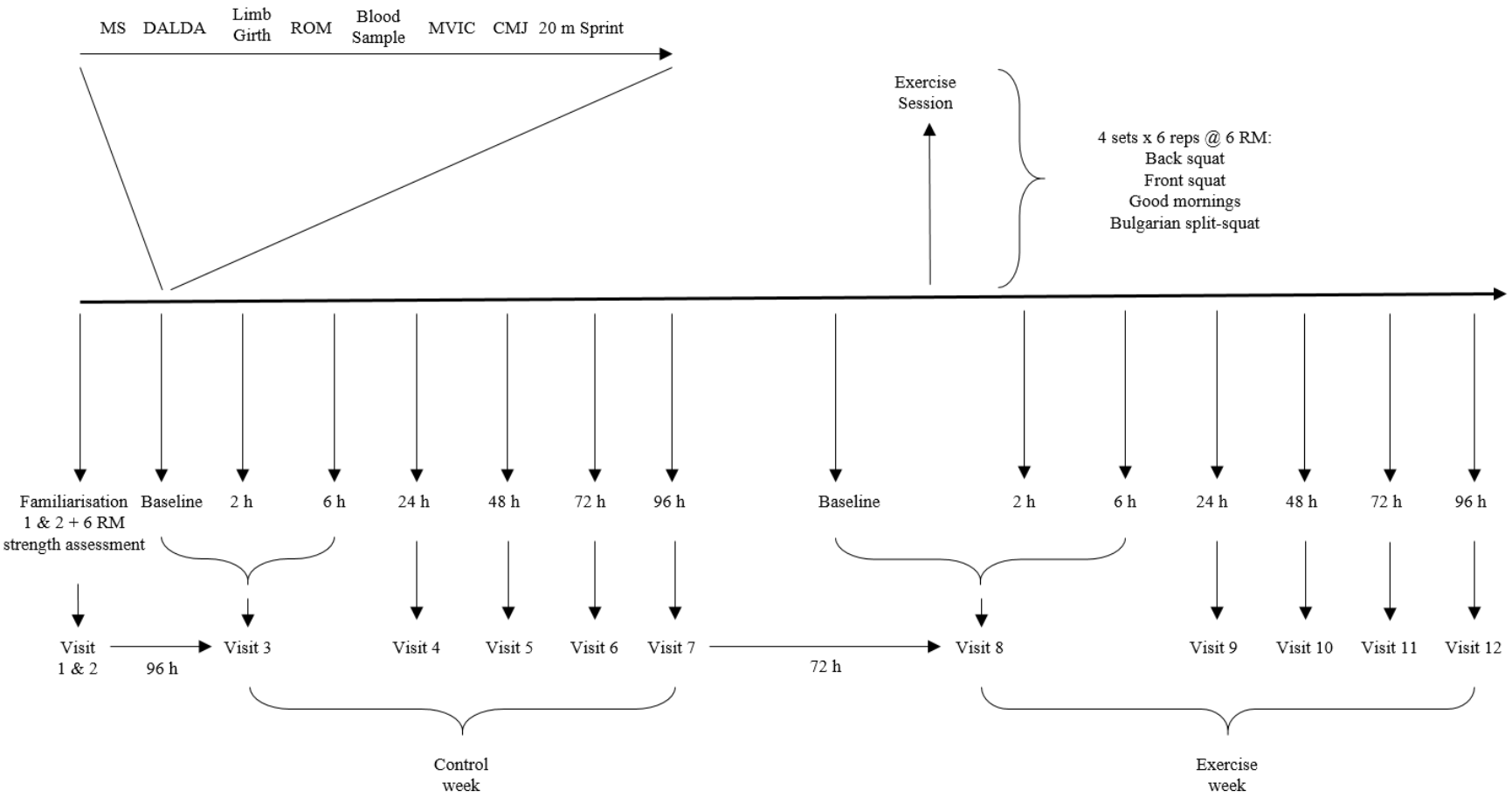

Figure 4.1 Schematic overview of the study timeline. The order of assessments is depicted for the Baseline session of the control week which was replicated for all subsequent time points. MS, muscle soreness; DALDA, daily analysis of life demands for athletes; ROM, range of motion; MVIC, maximal voluntary isometric contraction; CMJ, countermovement jump; RM, repetition maximum

Participants were instructed to maintain their habitual diet throughout the study and were asked to record their nutritional intake from visits 3 to 7 and then repeat this from visits 8 to 12. Participants were required to avoid the following throughout the study: exercise external to the protocol, any therapeutic interventions or nutritional supplements, alcohol, caffeine, and non-steroidal anti-inflammatory drugs. Additionally, participants were asked to refrain from food consumption in the two hours prior to any testing procedures.

4.2.3 Strength Assessment

For 6 RM strength assessment, please refer to section 3.4.1.

4.2.4 Resistance Exercise Session

For details of the resistance exercise session, please refer to section 3.5.

4.2.5 Maximal Voluntary Isometric Contraction

For procedures related to MVIC assessment, please refer to section 3.8.1.

4.2.6 Countermovement Jump

Participants were instructed to stand with their feet shoulder width apart on a force plate (AccuPower, AMTI, Massachusetts, USA) (Walsh, Ford, Bangen, Myer, & Hewett, 2006), with hands placed on hips. A standardised warm-up was performed consisting of countermovement jumps (CMJ) at 50, 75 and 90% of perceived maximum jump height. Participants then performed three maximal countermovement jumps and were instructed to maximise jump height by using their own choice of depth and pace, whilst maintaining hands on hips throughout and to land with straight legs. Prior to each attempt, the participant prepared themselves in the ready position and motionless on the force plate for at least 1 second to obtain bodyweight and the baseline period. Initiation of the contraction was identified as the first force value less than 5 SD of the baseline period (Chavda et al., 2018). If flight time was exaggerated by participants removing hands from the hips or bending their legs whilst in the air, the test was ordered to be performed again. Sixty seconds rest was afforded between attempts. The sample frequency for data collection was 400 Hz and variables were calculated using a published spreadsheet (Chavda et al., 2018), with jump height (cm) recorded and the best attempt used for subsequent analysis. Additionally, peak force (CMJ_{PF}) and rate of force development (RFD) were reported for the best attempt and RFD was calculated as the change in force ($N \cdot s^{-1}$) following the onset of the ascent phase of the CMJ in the time intervals 0-50 ms ($RFD_{0\text{-}50}$), 0-100 ms ($RFD_{0\text{-}100}$), 0-200 ms ($RFD_{0\text{-}200}$), 50-100 ms ($RFD_{50\text{-}100}$) and 100-200 ms ($RFD_{100\text{-}200}$). Previous research has reported RFD to differentiate the mechanisms of force loss following eccentric actions depending on the time window studied (Peñailillo et al., 2015) and may be a more sensitive indicator of neuromuscular

changes than MVIC force (Maffiuletti et al., 2016). While the rate at which force is developed is also dependent upon the type of muscle action (Tillin et al., 2012).

4.2.7 20 m Sprint

Following a warm-up with 20 m runs at 50, 75 and 90% of perceived maximal speed, participants performed three maximal 20 m sprints from a crouched sprinting position, starting 0.3 m behind the start line and leading with the same starting leg for all attempts. Sixty seconds rest was provided between each attempt which were timed using infrared timing gates (Smartspeed, Fusion Sport, Manchester, UK).

4.2.8 Active Muscle Soreness

For details of the muscle soreness assessment, please refer to section 3.9.

4.2.9 Daily Analysis of Life Demands for Athletes Questionnaire

At each time point throughout the study, participants completed the DALDA questionnaire (Rushall, 1990). The DALDA is divided into parts A and B, which represent the sources of stress and the manifestation of this stress in the form of symptoms, respectively. Part B was used for subsequent analysis based upon previous research showing this aspect was sensitive to determine fatigue and recovery during a period of intensified training (Coutts et al., 2007).

4.2.10 Limb Girth

The girth of the dominant limb was measured at the mid-point on the thigh using a flexible anthropometric tape while the participant stood relaxed in the anatomical zero position. Mid-point on the thigh was determined as the halfway point between the anterior superior iliac spine and the proximal aspect of the patella (MacDonald et al., 2014). The mid-point was marked to ensure consistent measurements during subsequent testing procedures. The mean of three measurements was used for subsequent analysis and intra-rater reliability was 0.29%.

4.2.11 Range of Motion

Range of motion (ROM) was determined as the difference between the joint angles of maximal voluntary flexion and extension of the knee joint (Chen et al., 2011). Standing in a position of anatomical zero, participants were instructed to elevate and straighten their leg as much as possible at which point the investigator measured the maximally extended angle using a goniometer (EZ Read Jamar Goniometer, Patterson Medical, Illinois, USA). Participants were then instructed to try and touch the hip with the heel whilst maintaining a position where both knees were held together. The investigator measured the maximally flexed angle and recorded this as ROM. All assessments were performed on the dominant limb with the non-dominant limb being used to stabilise the position. The mean of three measurements was used for subsequent analysis and intra-rater reliability was 2.4%.

4.2.12 Sleep Analysis

Participants wore wristwatch actigraphy monitors (wGT3X-BT Monitor, ActiGraph, LLC, FL, USA) to objectively assess sleep parameters alongside self-report sleep diaries. Participants were administered with the monitors following the baseline session and returned the devices following the 96 h session, therefore totalling four 24 h collection periods. The monitor was set to record physical activity using a three-dimensional accelerometer at a sampling rate of 30 Hz which was stored in 1 min epochs. Participants were required to wear the actigraphy monitors on the non-dominant wrist and were instructed to wear the device at all times throughout the study period, except when showering. The self-report sleep diary required participants to record sleep start and end times for each sleep period which were used to determine the start and end of the period analysed (Halson et al., 2014). All sleep periods (including naps) were summed to give total sleep for a 24 h period which was used for subsequent analysis (ActiLife Data Analysis Software, Version 6.11.6, ActiGraph, LLC, FL, USA). The estimation of sleep/wake duration from the actigraphy monitors was determined using a process reported elsewhere (Sargent et al., 2016). The following measures were subsequently calculated, which have been reported to show good agreement with polysomnography when analysed from actigraphy monitors (Sargent et al., 2016):

- Total sleep time (min): the sum of all periods classified as sleep in a given 24 h.
- Wake After Sleep Onset (WASO) (min): the sum of all periods classified as wake between the self-report start and end sleep times.
- Sleep efficiency (%): the percentage of time in bed that was spent asleep.
- Sleep latency (min): the difference between sleep onset time (determined by the ActiLife Data Analysis Software) and sleep start time (defined by participant self-report diary).

Additionally, participants were required to record a subjective rating for each sleep period on a 10-point Likert scale, with 1 being ‘worst possible sleep’ and 10 being ‘best possible sleep’, adapted from previous research (Lastella et al., 2015).

4.2.13 Blood Sample Collection and Analysis

Venous blood was collected as outlined in section 3.10. The time of collection for all blood markers was based upon likely known time-course responses (Table 4.1).

4.2.13.1 Creatine Kinase

For analysis of CK, please refer to section 3.10.1.

4.2.13.2 High Sensitivity C-Reactive Protein

For analysis of hsCRP, please refer to section 3.10.2.

4.2.13.3 Interleukin-6

For analysis of IL-6, please refer to section 3.10.3.

4.2.13.4 Interleukin-10

For analysis of IL-10, please refer to section 3.10.3.

4.2.13.5 Matrix Metalloproteinase-9

For analysis of MMP-9, please refer to section 3.10.4.

4.2.13.6 Skeletal Troponin I Fast Form

Serum skeletal troponin I fast form (sTnI) was determined by an ELISA using commercially available kit (Elabscience, Maryland, USA).

4.2.13.7 Protein Carbonyls

Serum PC samples were initially tested for their protein concentration using a Bradford Assay (Pierce™ Coomassie; Thermo Scientific, Maryland, USA). The results of which demonstrated that samples required a dilution factor of 1:5000 to fall within the recommended 10 μg/mL range for determination of PC using a commercially available ELISA kit (Oxiselect™; Cell Biolabs, Inc., California, USA).

4.2.13.8 Lipid Hydroperoxides

The ferrous iron/xylenol orange (FOX) assay (Wolff, 1994) was used to quantify the susceptibility to iron-induced LOOH formation in blood, as a measure of exercise-induced lipid peroxidation. Given the presence of iron ions in the assay protocol, higher LOOH values may be reported compared to other methods (Clifford et al., 2016). Absorbance was read at 560 nm using a spectrophotometer (U-2001, Hitachi, Berkshire, UK) (range 0-5 $\mu mol \cdot L^{-1}$).

4.2.13.9 Ascorbyl Free Radical

Electron paramagnetic resonance (EPR) spectroscopy was used to quantify the formation of ascorbyl free radical in blood using a Bruker EMX series X-band EPR spectrophotometer (Bruker, Karlsruhe, Germany). Briefly, 1 mL plasma and 1 mL dimethyl sulfoxide (DMSO) were mixed and slowly flushed into an aqua X multiple bore cavity cell. The EMX parameter settings were as follows; frequency, 9.785 GHz; microwave power, 20 mW; modulation frequency, 100 kHz; and modulation amplitude, 1.194 G. All EPR spectra underwent 3 scans, which were subsequently analysed, following the application of an 11-point filter, using WinEPR software (Version 3.2, Bruker WinEPR, Coventry, UK). The average spectral peak-to-trough line amplitude was used to determine free radical concentration.

Table 4.1 List of blood markers measured at specific time points.

Marker	Baseline	2h	6h	24h	48h	72h	96h
sTnI	✓	✓	✓	✓	✓	✓	✓
MMP-9	✓	✓	✓	✓	✓	✓	✓
IL-6	✓	✓	✓	✓			
IL-10	✓	✓	✓	✓			
CK	✓			✓	✓	✓	✓
hsCRP	✓			✓	✓	✓	✓
PC	✓	✓	✓	✓	✓		
LOOH	✓	✓	✓	✓	✓		
Ascorbyl	✓	✓	✓	✓	✓		

sTnI, skeletal troponin I fast form; MMP-9, matrix metalloproteinase-9; IL-6, interleukin-6; IL-10, interleukin-10; CK, creatine kinase; hsCRP, high sensitivity c-reactive protein; PC, protein carbonyls; LOOH, lipid hydroperoxides; Ascorbyl, ascorbyl free radical.

4.2.14 Statistical Analysis

4.2.14.1 Reliability and Signal-to-Noise-Ratio

Typical error was calculated for reliability (Hopkins, 2000) during the control week and presented as coefficient of variation (%) using a published spreadsheet (Hopkins, 2015). Range of motion, DALDA and sleep rating values are presented as absolute values. Signal-to-noise ratio refers to the magnitude of the largest mean effect observed between the exercise week and control week at any time point, divided by the typical error calculated from the control week (Buchheit, 2014).

4.2.14.2 Magnitude of change and time course of the acute physiological response

Measures with a signal-to-noise ratio of greater than 1.5 were then analysed by making probabilistic magnitude-based inferences, described in more detail in section 3.11. A change greater than 1.5 times the typical error was selected as the threshold based upon the suggestion that this can be considered a real change as opposed to noise associated with within-subject variation (Hopkins, 2000). Magnitude-based inferences build on the signal-to-noise ratio as this method takes into account the individual responses, as demonstrated by the confidence intervals, as well as the smallest worthwhile change before making the final interpretation (Hopkins et al., 2009; Hopkins & Batterham, 2016). Given the large number possible, comparisons were made between baseline and each subsequent time point (i.e. baseline-2 h,

baseline-6 h, baseline-24 h, baseline-48 h, baseline-72 h, baseline-96 h) with differences compared between the control and exercise weeks. Values for the DALDA were not log-transformed and are presented as absolute values. Baseline values of the control and exercise weeks were analysed for differences using a published spreadsheet (Hopkins, 2006).

4.3 Results

4.3.1 Reliability and Signal-to-Noise-Ratio

Table 4.2 shows the typical error and signal-to-noise ratio for all measures. Based on the signal-to-noise ratio, the most sensitive measures were MVIC, 20 m sprint, CMJ_{PF}, $RFD_{100-200}$, muscle soreness, DALDA Part B, limb girth, MMP-9, IL-6, CK, hsCRP and ascorbyl free radical which all had a ratio of >1.5. All the other measures were therefore not reliable enough or did not change sufficiently post-exercise to be sensitive to detect change in acute physiological responses.

4.3.2 Magnitude of change and time course of the acute physiological response

Baseline values were *possibly* greater for the exercise week compared to the control week for MVIC force (small effect), *likely* greater for CMJ_{PF} (moderate effect), and *possibly* smaller for hsCRP (small effect). All other effects between baseline values were *unclear.*

4.3.2.1 Maximal Voluntary Isometric Contraction

Baseline values for MVIC force were 242 ± 55 N and 264 ± 60 N for the control and exercise weeks, respectively. There was a *most likely* moderate reduction in maximal muscle force generating capacity following the resistance exercise session at 2 h post-exercise (−19.3; ± 5.7%; Figure 4.2). The decrease in force production was *very likely* moderate at 6 h (−13.1; ± 6.1%), *most likely* moderate at 24 h (−12.9; ± 3.0%), *likely* small at 48 h (−7.8; ± 7.9%) and *possibly* small at 96 h post-exercise (−6.9; ± 9.0%) compared to the control week. Effects were *unclear* at 72 h post-exercise (−4.8; ± 10.8%).

4.3.2.2 20 m Sprint Performance

Baseline 20 m sprint times were 3.15 ± 0.10 s and 3.15 ± 0.06 s for the control and exercise weeks, respectively. An impairment in sprint performance was *very likely*, demonstrated by an increased 20 m sprint time at 2 h (large effect, 4.2; ± 2.3%) and 6 h post-exercise (moderate effect, 3.0; ± 1.8%) as well as *likely* small increases in sprint time at 24 h (1.6; ± 2.1%) and 48 h post-exercise (1.1; ± 1.3%) compared to the control week (Figure 4.2). There was an *unclear* outcome at 72 h (0.9; ± 1.8%), while sprint time was *possibly* decreased at 96 h post-exercise (small effect, −0.8; ± 1.8%).

Table 4.2 Values for the typical error and signal-to-noise ratio for a range of measures.

Measure	TE (absolute)	TE (%)	Signal-to-noise ratio	ICC
Muscle soreness	2.3%	72%	15.0	0.66 ± 0.22
20 m sprint	0.03 s	1.1%	3.8	0.92 ± 0.07
MVIC	14 N	5.4%	3.6	0.96 ± 0.03
Creatine Kinase	150 $U \cdot L^{-1}$	28%	3.5	0.94 ± 0.05
CMJ_{PF}	75 N	6.6%	3.1	0.63 ± 0.23
Limb girth	0.3 cm	0.5%	2.2	0.99 ± 0.01
Ascorbyl Free Radical	59 351 AU	12%	2.2	0.97 ± 0.02
hsCRP	0.20 $mg \cdot L^{-1}$	35%	2.0	0.94 ± 0.05
$RFD_{100-200}$	150 $N \cdot s^{-1}$	24%	1.9	0.90 ± 0.07
DALDA Part B	0.9	N/A	1.7	0.89 ± 0.08
IL-6	0.46 $pg \cdot mL^{-1}$	114%	1.7	0.20 ± 0.10
MMP-9	38 925 $pg \cdot mL^{-1}$	18%	1.6	0.83 ± 0.11
Sleep latency	5.2 min	149%	1.5	N/A
Sleep rating	0.6	N/A	1.3	N/A
RFD_{0-200}	103 $N \cdot s^{-1}$	21%	1.2	0.93 ± 0.05
Jump height	3.6 cm	12%	0.8	0.63 ± 0.23
Total sleep time	38 min	10%	0.8	N/A
sTnI	29 $pg \cdot mL^{-1}$	34%	0.8	0.80 ± 0.13
RFD_{50-100}	168 $N \cdot s^{-1}$	41%	0.8	0.87 ± 0.09
IL-10	2.7 $pg \cdot mL^{-1}$	17%	0.7	1.00 ± 0.01
RFD_{0-100}	106 $N \cdot s^{-1}$	29%	0.7	0.91 ± 0.06
Sleep efficiency	5.4%	N/A	0.6	N/A
Protein Carbonyls	1.1 $nmol \cdot mg^{-1}$	21%	0.6	0.83 ± 0.12
Range of motion	2.6°	N/A	0.5	0.91 ± 0.06
RFD_{0-50}	179 $N \cdot s^{-1}$	59%	0.5	0.71 ± 0.20
WASO	21 min	39%	0.4	N/A
Lipid Hydroperoxides	0.18 $mmol \cdot mL^{-1}$	12%	0.3	0.75 ± 0.16

TE, Typical Error; ICC, Intraclass correlation coefficient; MVIC, Maximal Voluntary Isometric Contraction; CMJ_{PF}, countermovement jump peak force; RFD, Rate of Force Development; DALDA, Daily Analysis of Life Demands for Athletes; WASO, Wake After Sleep Onset; sTnI, skeletal troponin fast form; MMP-9, matrix metalloproteinase-9; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IL-10, interleukin-10. Solid line represents threshold for the signal-to-noise ratio (Buchheit, 2014), with measures above the line taken forward for subsequent analysis (Hopkins, 2000).

4.3.2.3 Countermovement Jump

At baseline, CMJ_{PF} was 1059 ± 103 N and 1212 ± 209 N for the control and exercise weeks, respectively. There was a *most likely* large reduction in force at 2 h (−19; ± 6.2%) and 6 h (−20; ± 6.3%), *very likely* large decrease at 24 h (−20; ± 11%), 48 h (−19; ± 9.3%) and 72 h (−20; ± 14%) and *likely* moderate impairment at 96 h post-exercise (−14; ± 14%).

4.3.2.4 Rate of Force Development

Baseline values for $RFD_{100\text{-}200}$ were 582 ± 243 $N \cdot s^{-1}$ and 550 ± 315 $N \cdot s^{-1}$ for the control and exercise weeks, respectively. There was a *likely* small reduction in $RFD_{100\text{-}200}$ at 2 h (−22; ± 24%), *likely* moderate reduction at 24 h (−34; ± 24%), *very likely* moderate decrease at 48 h (−46; ± 25%) and a *likely* moderate decrease at 72 h (−43; ± 37%). Force production was *likely* moderately increased at 96 h (27; ± 33%), while effects at 6 h were *unclear* (−0.2; ± 33%).

4.3.2.5 Limb Girth

Baseline limb girth values were 61 ± 3 cm and 61 ± 3 cm for the control and exercise weeks, respectively. Effects at 2 h (0.6; ± 0.7%) and 6 h (0.4; ± 0.6%) post-exercise were *likely trivial* compared to the control week, respectively (Figure 4.2). There were *possible* increases in limb girth at 24 h (small effect, 0.9; ± 0.8%), 48 h (small effect, 1.1; ± 1.1%), 72 h (0.7; ± 0.8%) and 96 h (0.8; ± 0.7%) post-exercise compared to the control week.

4.3.2.6 Muscle Soreness

Baseline muscle soreness values were 9.0 ± 13.0% and 4.6 ± 4.4% for the control and exercise weeks, respectively. Muscle soreness was *very likely* increased at 2 h (large effect, 6.0; x/÷2.8), *most likely* increased at 6 h (large effect, 5.4; x/÷2.2) and 24 h (large effect, 10.3; x/÷2.8), *very likely* increased at 48 h (very large effect, 12.4; x/÷5.1) and 72 h (large effect, 8.4; x/÷3.3), and *likely* increased at 96 h post-exercise (moderate effect, 2.7; x/÷2.6) compared to the control week (Figure 4.3).

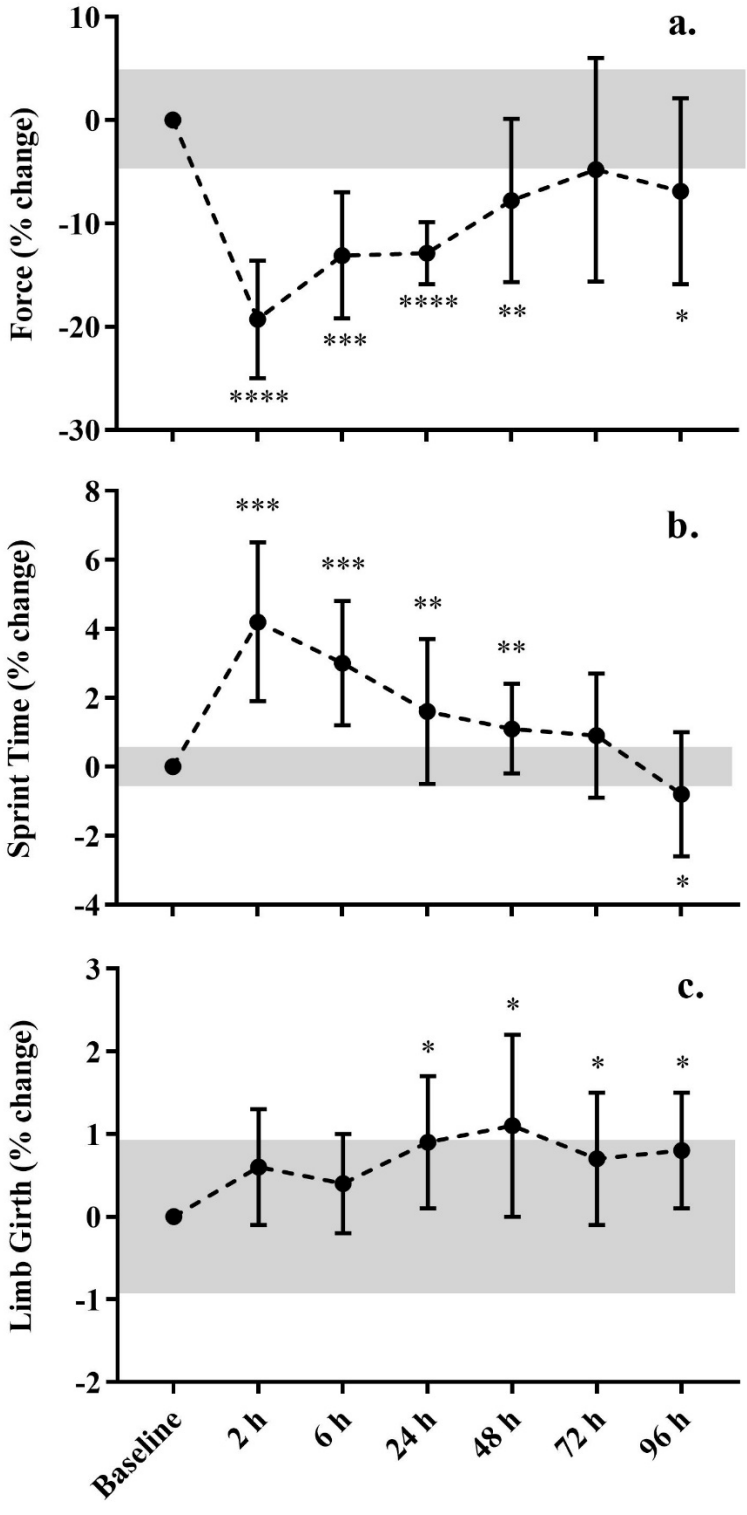

Figure 4.2 Percentage change from baseline in (a) maximal voluntary isometric contraction force, (b) 20 m sprint time and (c) limb girth. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

4.3.2.7 Daily Analysis of Life Demands for Athletes

At baseline, participants reported 2 ± 2 aspects of the DALDA Part B as 'worse than normal' during both the control and exercise weeks. There was a *likely* increase in the number of 'worse than normal' ratings at 2 h (small effect, 1; ± 1) and 6h (moderate effect, 1; ± 1), *very likely* increase at 24 h (moderate effect, 2; ± 1), and *likely* increase at 48 h (moderate effect, 1; ± 1) and 72 h (small effect, 1; ± 1) post-exercise compared to the control week (Figure 4.3). There was a *likely* decrease in the number of 'worse than normal' ratings at 96 h post-exercise (small effect, −1; ± 1) compared to the control week.

4.3.2.8 Blood Analysis

Baseline MMP-9 concentrations were 199 781 ± 113 892 pg·mL^{-1} and 175 586 ± 54 072 pg·mL^{-1} for the control and exercise weeks, respectively. There was a *very likely* moderate increase in MMP-9 concentrations at 2 h post-exercise (29.3; ± 21.4%) compared to the control week (Figure 4.4). Effects were *unclear* at 6 h (15.3; ± 39.6%), *possibly* decreased at 24 h (−4.0; ± 26.6%) and *unclear* at 48 h (−1.9; ± 27.0%), 72 h (6.5; ± 29.3%) and 96 h post-exercise (10.6; ± 31.0%).

The concentration of IL-6 was 1.05 ± 0.44 pg·mL^{-1} and 0.95 ± 0.22 pg·mL^{-1} at baseline for the control and exercise weeks, respectively. There was a *very likely* increase in IL-6 at 2 h (moderate effect, 1.51; x/÷1.37) and 6 h post-exercise (very large effect, 2.98; x/÷2.39) compared to the control week (Figure 4.4). There was a *possible* decrease in IL-6 at 24 h post-exercise (0.98; x/÷1.25) compared to the control week.

Baseline CK concentrations were 408 ± 707 U·L^{-1} and 312 ± 422 U·L^{-1} for the control and exercise weeks, respectively. Exercise resulted in a *very likely* moderate increase in CK concentrations at 24 h (2.0; x/÷1.6) and a *likely* small increase at 48 h (1.5; x/÷1.6) post-exercise compared to the control week (Figure 4.4). Effects were *unclear* at 72 h (1.2; x/÷1.5), while there was a *possible* decrease at 96 h post-exercise (0.8; x/÷1.7) compared to the control week.

The concentration of hsCRP was 0.64 ± 0.55 mg·L^{-1} and 0.40 ± 0.27 mg·L^{-1} for the control and exercise weeks, respectively. Concentrations of hsCRP were *very likely* increased at 24 h (small effect, 1.7; x/÷1.3), as well as there being a *likely* small increase at 48 h (1.4; x/÷1.5) and 72 h post-exercise (1.7; x/÷1.5) compared to the control week (Figure 4.4). Effects were *unclear* at 96 h post-exercise (1.5; x/÷1.9).

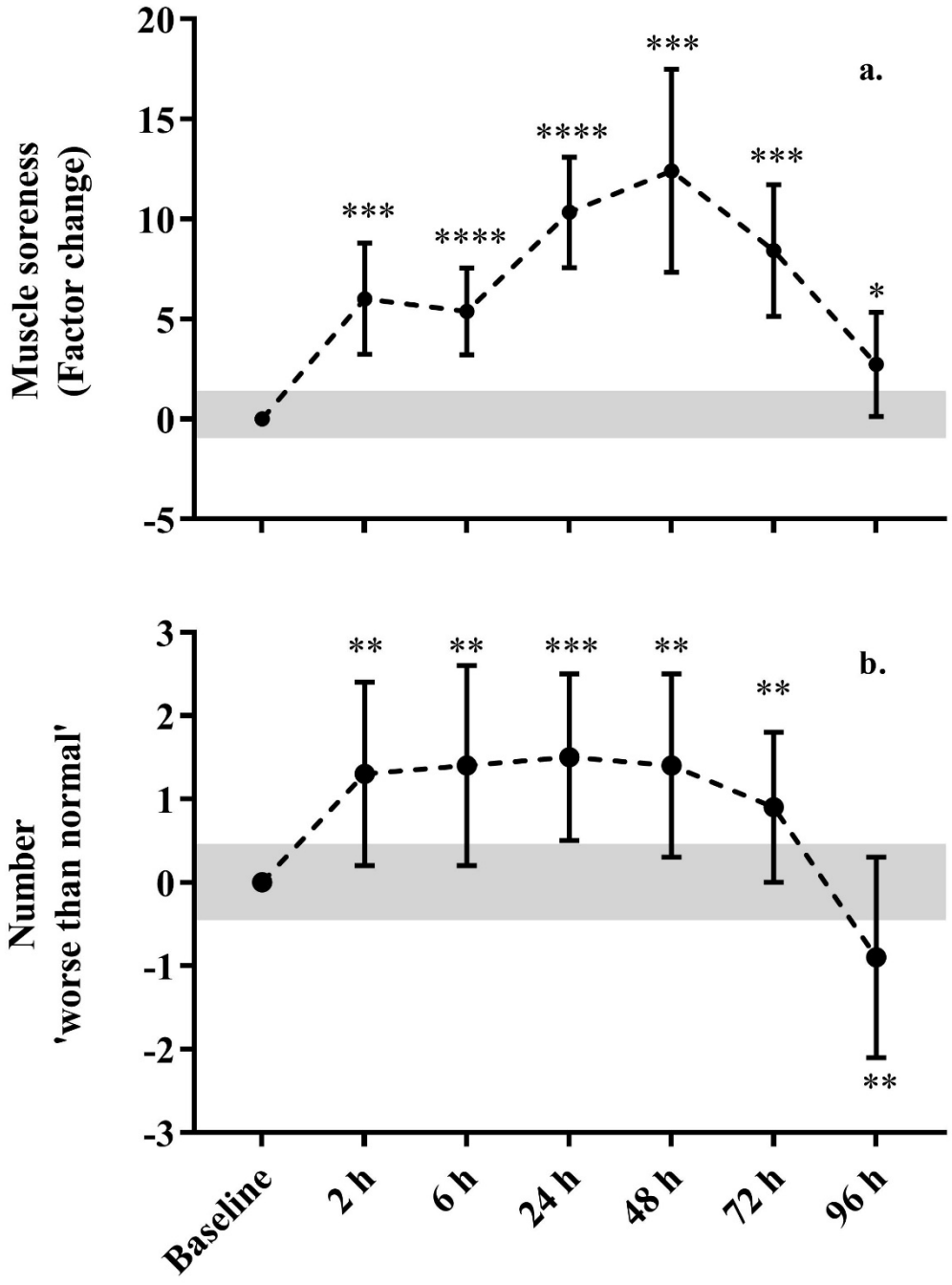

Figure 4.3 Change from baseline in (a) muscle soreness and (b) Daily Analysis of Life Demands for Athletes. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

Values for ascorbyl free radical were 436 425 ± 232 381 AU and 400 094 ± 199 382 AU at baseline for the control and exercise weeks, respectively. There was a *likely trivial* effect of exercise at 2 h post-exercise (1.1; x/÷1.3) compared to the control week. Effects were *unclear* at 6 h (0.9; x/÷2.4), 24 h (0.8; x/÷2.2) and 48 h post-exercise (0.7; x/÷2.1).

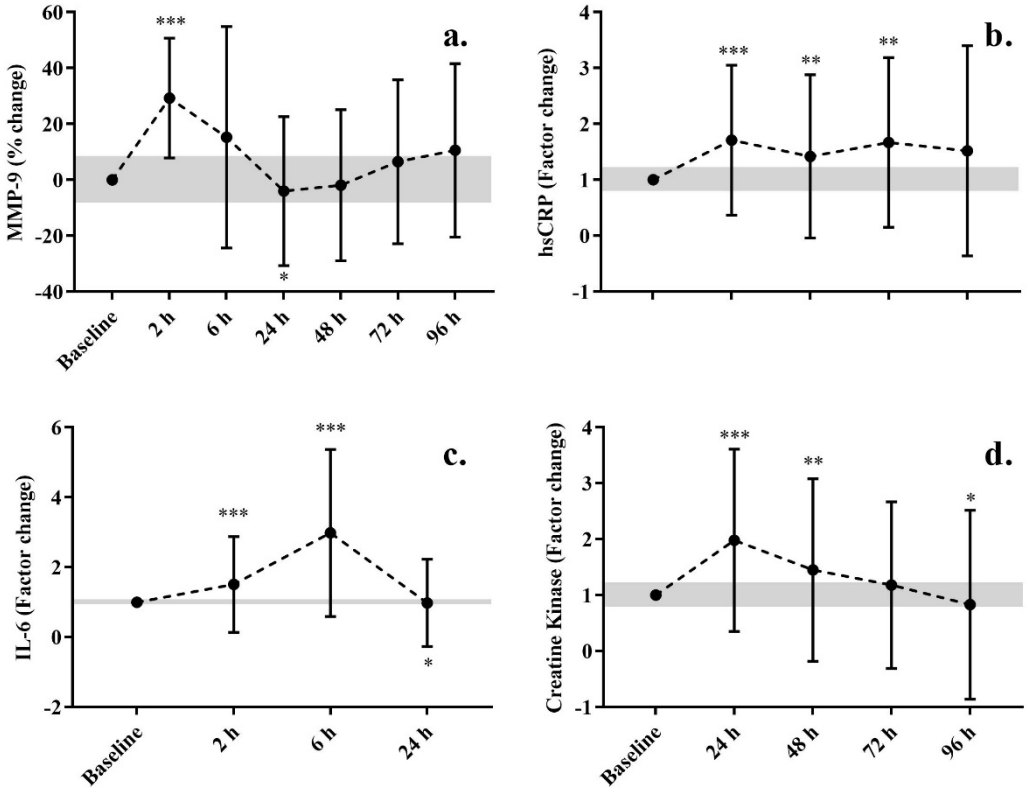

Figure 4.4 Change from baseline in (a) matrix metalloproteinase-9 (MMP-9), (b) high sensitivity C-reactive protein (hsCRP), (c) interleukin-6 (IL-6) and (d) creatine kinase concentrations. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely change

Table 4.3 Recommended sampling points for measures that exhibited clear effects.

Measure	2h	6h	24h	48h	72h	96h
MVIC	✓*	✓	✓	✓		
20 m sprint	✓*	✓	✓	✓		
CMJ_{PF}	✓	✓*	✓	✓	✓	✓
$RFD_{100-200}$	✓		✓	✓*	✓	✓
Limb girth			✓	✓*	✓	✓
Muscle soreness	✓	✓	✓	✓*	✓	✓
DALDA Part B	✓	✓	✓*	✓	✓	
Creatine Kinase			✓*	✓		
hsCRP			✓*	✓	✓	
IL-6	✓	✓*				
MMP-9	✓*					

MVIC, Maximal Voluntary Isometric Contraction; CMJ_{PF}, countermovement jump peak force; RFD, rate of force development; DALDA, Daily Analysis of Life Demands for Athletes; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; MMP-9, matrix metalloproteinase-9. * represents the peak change for a given measure.

4.4 Discussion

This study had two primary purposes: (i) to provide information as to the sensitivity of a variety of measures used as indirect measures to quantify the acute physiological response to resistance exercise, and (ii) to provide a profile to characterise the magnitude of change and time course of this response. We quantified these results in individuals accustomed to resistance exercise across a timescale common to research investigating acute physiological responses, allowing transferability of the results. The results of this study indicate that MVIC, 20 m sprint, CMJ_{PF}, $RFD_{100-200}$, muscle soreness, limb girth, DALDA Part B, MMP-9, IL-6, CK, hsCRP and ascorbyl free radical were sensitive measures to detect change in response to resistance exercise, with a signal-to-noise ratio of >1.5. Using these measures, clear effects were reported with aspects of the acute physiological response apparent as soon as 2 h, through to 96 h post-exercise, determined via magnitude-based inferences.

4.4.1 Reliability and signal-to-noise ratio

Following resistance exercise, muscle soreness was the measure most sensitive to detect change in trained individuals as demonstrated by a signal-to-noise ratio of 15.0. A variety of other measures also showed a signal-to-noise ratio of >1.5. The choice of the threshold as >1.5

was based upon recommendations by Hopkins (2000) in which a change can be considered real when it is greater than 1.5 times the typical error. Additionally, the typical error used in such situations should come from a reliability study of the same duration as the experiment in which there is no true change in the individual's measurements between trials (Hopkins, 2000). Therefore the design used in the present study conforms to these recommendations. Previous research using this method to track aerobic training adaptations reported exercise heart rate as the most sensitive measure to change with a signal-to-noise ratio of 1.6 (Buchheit, 2014). The measures in the present study therefore compare favourably.

All of the other measures assessed in this study either exhibited poor reliability or did not demonstrate a post-exercise change that was of great enough magnitude to provide a signal-to-noise ratio of >1.5. Taking LOOH and CK as examples, by just showing the typical error, it may be assumed that LOOH (12%) are a more sensitive marker in this scenario than CK (28%). However, once the magnitude of post-exercise change is considered, it becomes apparent that resistance exercise has a profound impact upon CK (98% increase), whilst LOOH are relatively unchanged (3.5% increase/decrease). Taking both the reliability and magnitude of change post-exercise into account with the signal-to-noise ratio, these results provide novel information as to the sensitivity for a range of measures used to assess acute physiological responses following resistance exercise.

In the current study, the signal-to-noise ratio was ≤1.5 for a range of measures suggesting either significant typical error during the control week, or a lack of change during the exercise week contributed to the outcome that these markers were not sensitive enough to detect changes in acute physiological responses. We report these findings in contrast to others that have shown resistance exercise to impact: jump height (Byrne & Eston, 2002a, 2002b) and ROM (Chen et al., 2011), as well as the concentrations of sTnI (Rankin et al., 2015), IL-10 (Hirose et al., 2004) and PC (Bowtell et al., 2011). Interestingly, $RFD_{100\text{-}200}$ was the only RFD time point sensitive to change, highlighting that even different aspects of the same measure may exhibit varying levels of usefulness. Differences in these findings compared to previous research may be attributed to either the training status of participants or the level of mechanical challenge imposed by the exercise bout such that these factors negated any impact upon these measures in the present study. Alternatively, these studies may have reported changes that were within the typical error of the measurement. It is noteworthy that this study utilised an exercise session designed to target the lower limbs and the upper body response may be different (Vernillo, Temesi, Martin, & Millet, 2018). The authors acknowledge that it is unfeasible to expect all researchers to conduct reliability studies prior to an investigation. Therefore this study provides information as to the reliability of a range of measures for use in future research, whilst incorporating important facets such as the use of a frequently used

cohort and matching the time scale between baseline and follow up assessments (Atkinson & Batterham, 2015).

4.4.2 Magnitude of change and time course of the acute physiological response

Resistance exercise impaired MVIC force and 20 m sprint performance which peaked at 2 h post-exercise with the effects still apparent for the ensuing days following the exercise bout. Clear effects on numerous physiological and perceptual measures further highlight the multitude of acute physiological responses. These findings support previous research which demonstrates a similar range of perturbations following resistance exercise in the hours and days post-exercise (Miles et al., 2008; Vincent & Vincent, 1997b). In the context of this study, measures such as MVIC/20 m sprint/CMJ_{PF}/$RFD_{100\text{-}200}$, MMP-9/IL-6/hsCRP and CK reflect disturbances to muscle function (Morton et al., 2005; Peñailillo et al., 2015), inflammatory processes (Miles et al., 2008; Peake, Nosaka, et al., 2005) and muscle cell membrane integrity (Brancaccio et al., 2007; Clarkson & Hubal, 2002), respectively.

Increased perceptions of muscle soreness occurred concomitantly with higher 'worse than normal' ratings on the DALDA Part B, suggesting feelings of soreness and stress are an important facet of the acute post-exercise period. Muscle soreness is a well-established physiological response to resistance exercise (Miles et al., 2008; Vincent & Vincent, 1997b). Here novel information is presented that Part B of the DALDA is sensitive to change in the acute period following a single bout of resistance exercise, increasing the potential application of this measure in addition to overtraining research (Coutts et al., 2007). Together these findings highlight sensitive measures that are able to detect changes to characterise the magnitude of change and time course of the acute physiological response to resistance exercise. Table 4.3 provides the recommended sampling points so that researchers/practitioners can capture meaningful changes in these measures.

Research has demonstrated that strategies designed to attenuate acute physiological responses can accelerate recovery processes (Bowtell et al., 2011; Rankin et al., 2015), whilst others suggest that these disturbances may form part of the normal response required as part of the adaptive process (Peake, Markworth, et al., 2015; Roberts et al., 2015). An understanding of the most sensitive measures as well as recommended sampling points provides key information for those seeking to identify the effectiveness of imposed strategies that look to manipulate acute physiological responses for the purposes of recovery and/or adaptation. Application of these results may also be of benefit for those profiling an individual's readiness to train.

Despite a signal-to-noise ratio of 2.2, the peak effect of resistance exercise on ascorbyl free radical was *unclear*. This may be explained by the significant variation in individual

responses, as demonstrated by large confidence intervals, which showed both a substantial increase and decrease in the concentration following the exercise bout. Therefore this may be considered a sensitive measure but would have required greater than eight participants to detect real changes following the resistance exercise bout in this study. However, results calculated in this way may only apply in the context of the selected study (Buchheit, 2014). Whether similar findings would be seen in the context of different forms of exercise or in a cohort of untrained individuals remains to be elucidated.

A potential limitation of the study is the difference in baseline values for MVIC force, CMJ_{PF} and hsCRP. It is possible that the baseline measures of the control week may have been impacted by the pre-testing 6 RM strength assessment conducted 96 h prior. However, MVIC force and hsCRP had returned to baseline at 96 h following the exercise intervention, which would be expected to demonstrate a greater response compared to the 6 RM strength assessment, and along with all other measures showing *unclear* effects between baseline values, the likelihood of this is reduced. Instead this perhaps highlights learning effects (performance measures) and biological variation (blood markers) that may exist despite familiarisation and control measures which further highlights the value of this data in providing the typical error of measures across an appropriate timescale.

In summary, present findings show that MVIC force, 20 m sprint performance, CMJ_{PF}, $RFD_{100\text{-}200}$, limb girth, muscle soreness, DALDA Part B, MMP-9, IL-6, CK and hsCRP are useful measures to detect meaningful change in acute physiological responses to resistance exercise in trained individuals. When characterising the magnitude of change and time course of this response, the present study identifies recommended sampling points for future research. The application of this data is valuable to researchers and practitioners looking to investigate the effectiveness of strategies that manipulate acute physiological responses in order to optimise recovery processes and/or training adaptation as well as strength and conditioning coaches profiling an individual's readiness to train.

4.5 Perspectives

This chapter addressed the first and second aims of the thesis, to *'Identify sensitive measures and recommended sampling points for acute physiological responses following resistance exercise'*, and to *'Provide a profile to characterise the magnitude of change and time course of the acute physiological response following resistance exercise'*. This study found several sensitive measures of the acute physiological response that exhibited clear effects following resistance exercise. Additionally, information was provided as to the magnitude of change and time course of the response to highlight recommended sampling points. Together, the

measures and sampling points identified can be used to address the aims of subsequent chapters.

The findings of this study are the first to holistically investigate the usefulness of acute physiological response measures in a real-world scenario. This includes: recruiting a trained cohort, employing an applied exercise session and taking measures across a timescale relevant to the research area. This is of importance to applied sports scientists who wish to use measures of acute physiological responses to monitor an athlete's readiness to train or see the effect of a recovery strategy.

These results are also the first to examine the usefulness of acute physiological response measures using a within-subject, crossover design which enabled both the typical error to be ascertained during the non-exercise control week and the magnitude of change with resistance exercise during the exercise week. Given the inter-individual and diurnal variation associated with these measures, the results of this study therefore identify measures that can demonstrate a clear 'signal' above the 'noise' of the typical error.

The useful measures and recommended sampling points identified in the present chapter may therefore be applied within future research. Specifically, to investigate the impact of strategies designed to manipulate acute physiological responses for the purposes of recovery. Accordingly, the following chapter will investigate the effect of HWI on acute physiological responses following resistance exercise.

5 Effect of Hot Water Immersion on Acute Physiological Responses Following Resistance Exercise

This study investigated the effect of HWI on acute physiological responses and recovery following resistance exercise. The main findings demonstrated that HWI is a viable means of heat therapy that can support a greater intramuscular temperature following resistance exercise. The elevated intramuscular temperature may have manipulated inflammatory processes. Although changes in other acute physiological response markers were independent of changes in intramuscular temperature associated with HWI. These results represent the first investigation into the acute physiological responses of a 'real-world' HWI protocol following resistance exercise, alongside the use of a trained cohort, applied exercise session and utilising good nutritional practice.

5.1 Introduction

The previous chapter of this thesis identified a range of useful measures and recommended sampling points for assessing acute physiological responses following resistance exercise. To build on these findings within the main theme of this thesis, the current chapter will introduce HWI and investigate its effects on acute physiological responses following resistance exercise, whilst employing the recommended measures and time points of assessment from the first chapter.

Post-exercise hydrotherapy is common practice among athletic individuals, with the goal of enhancing acute recovery following training and competition (Vaile et al., 2010). While cold water immersion (CWI) has received growing attention in the literature, there is a paucity of research investigating the effectiveness of HWI on exercise recovery in humans, with equivocal findings to date (McGorm et al., 2018). The efficacy of such strategies is likely related to the specific recovery needs of the individual (Minett & Costello, 2015), therefore a greater understanding of the impact of HWI on acute physiological responses will aid in the application of this strategy in exercise recovery.

Hot water immersion is thought to exert a physiological impact primarily through an increase in cutaneous and subcutaneous tissue temperature which induces peripheral vasodilation and a subsequent increase in blood flow (Wilcock et al., 2006). The ensuing increase in permeability of cellular, lymphatic and capillary vessels may drive an increased rate of metabolism, nutrient delivery and clearance of waste products (Baker, Robertson, & Duck, 2001; Coté et al., 1988), which could aid exercise recovery. The research behind these proposed mechanisms has typically occurred in the field of physiotherapy or with techniques of heat application including ultrasound and heat packs (Bonde-Petersen et al., 1992; Knight & Londeree, 1980; Wyper & McNiven, 1976). Whether similar effects would be seen with HWI and following an exercise session which stimulates acute physiological responses typical of a 'real world' training session requires further investigation.

Despite the limited insights into the mechanisms that would rationalise enhanced recovery in an exercise setting, research from human studies using post-exercise heating has produced some promising evidence (McGorm et al., 2018; Vaile et al., 2010). Cheng et al. (2017) reported that acute recovery following exhaustive intermittent arm cycling is influenced by intramuscular temperature, with mean power output better preserved after the upper limbs were heated to ~38°C compared to being cooled to ~15°C for 2 h post-exercise. Based upon replication of these findings in an animal model, the authors attributed the enhanced recovery to better rates of glycogen resynthesis with heating versus cooling, due to the increased tissue temperature promoting rates of enzymatic processes (Cheng et al., 2017). Others have also

reported HWI to improve the recovery of strength (Clarke, 1963; Vaile et al., 2008b) and power (Viitasalo et al., 1995) following fatiguing isometric exercise, a leg press protocol designed to elicit delayed onset-muscle soreness and throughout a strength/power training week for track and field athletes, respectively. Enhancing recovery in this way would be of benefit to those performing resistance exercise as part of a progressive programme, however the effect of HWI on these measures following an ecologically valid exercise session are yet to be determined.

The effect of post-exercise heating on other acute physiological responses is inconclusive. Following high-intensity intermittent exercise (Pournot et al., 2011) and an intense strength/power training week (Viitasalo et al., 1995), HWI did not influence the appearance of intramuscular enzymes in the blood. Whereas after a single bout of eccentric exercise, Vaile et al. (2008b) reported a reduction in plasma creatine kinase with HWI. Heat therapy may reduce pain via an analgesic effect on nerves (Baker et al., 2001) and has been shown to reduce soreness following lumbar extension exercise (Mayer et al., 2006). Conversely, other reports have demonstrated post-exercise heating to exert no impact upon muscle soreness (Kuligowski et al., 1998; Pournot et al., 2011; Vaile et al., 2008b; Viitasalo et al., 1995). Differences in exercise modality, HWI protocol and timing of recovery measures make conclusions problematic, lending to the assertion that further research utilising ecologically valid protocols are required in this area (McGorm et al., 2018; Vaile et al., 2010).

A disparity currently exists between the hypothesised benefits of post-exercise heating and the evidence for HWI to improve acute exercise recovery (McGorm et al., 2018; Vaile et al., 2010). Given the growing use of thermotherapy in recreationally active and athletic populations, further insights are required to provide evidence-based rationale supporting or refuting the application of HWI as a recovery aid. Therefore, the aim of the present study was to investigate the effect of HWI on a range of acute physiological responses following resistance exercise in a trained cohort. Recommended acute physiological response measures and time points of assessment will be used from the first chapter, as well as recruiting participants from the same criteria and employing an identical exercise session.

5.2 Methods

5.2.1 Participants

Sixteen strength trained males volunteered to take part in the study (Table 5.1). The study was granted ethical approval (application number: 1686) by the London Sports Institute Ethics Sub-Committee at Middlesex University.

Table 5.1 Participant characteristics.

	Intervention group	
	PAS	HWI
Age (yrs)	24 ± 4	25 ± 4
Height (m)	1.77 ± 0.05	1.80 ± 0.07
Body mass (kg)	88 ± 17	89 ± 14
Surface area:body mass	32 ± 3	32 ± 4
1 RM (kg)	158 ± 30	158 ± 31

PAS, passive recovery group; HWI, hot water immersion group; 1 RM, 1 repetition maximum. Unless indicated, effects between groups were *unclear*.

5.2.2 Experimental Design

Using a between-subject design, participants were pair matched for baseline strength (1 repetition maximum [RM] back squat) and body composition (body surface area to body mass ratio) and assigned to HWI or passive recovery (PAS) groups. Previous research has shown the relationship between body surface area relative to body mass to be an important influencer on thermal and physiological responses to hydrotherapy (Stephens et al., 2014). Participants attended the laboratory on four occasions (Figure 5.1). During the first visit, anthropometric data was collected (height and body mass), before participants were familiarised with experimental procedures. Additionally, participants performed a strength assessment to determine a 6 RM for the exercise techniques used in the exercise session (back squat, front squat, good morning, Bulgarian split-squat). The second visit involved a body composition assessment prior to a further familiarisation with experimental procedures. Participants then performed a strength assessment to determine a 1 RM for the back squat.

Visit 3 formed the start of the experimental period and required participants to perform baseline assessments in the following order: muscle soreness, blood sample, intramuscular temperature, maximal voluntary isometric contraction (MVIC) and an isometric squat (ISO). Following baseline assessments, participants performed a resistance exercise session and then completed either the HWI or PAS interventions. Intramuscular temperature assessments were repeated: post-exercise, post-intervention, 1 h and 2 h post-exercise. Muscle soreness, blood sample, MVIC and ISO were repeated at 2 h post-exercise. During visit 4, participants returned to the laboratory to perform 24 h post-exercise assessments for muscle soreness, blood sample, MVIC and ISO.

Prior to visit 3, participants were provided with a standardised meal (27 g porridge oats, 180 mL semi-skimmed milk, 200 g high protein yoghurt) to consume 2 h before arrival at the laboratory. Participants also consumed a ready to drink protein milk (30 g protein) following completion of the exercise session and after the 2 h post-exercise assessments, between which they were required to be fasted (Roberts et al., 2015). Additionally, participants were asked to refrain from food consumption in the two hours prior to any testing procedures. Aside from the control measures, participants were instructed to maintain their habitual dietary intake throughout the study. Participants were required to avoid the following throughout the study: exercise external to the protocol, any therapeutic interventions or nutritional supplements, alcohol and non-steroidal anti-inflammatory drugs.

5.2.3 6 Repetition Maximum

For details of the 6 RM assessment, please refer to section 3.4.1.

5.2.4 1 Repetition Maximum

For details of the 1 RM assessment, please refer to section 3.4.2.

5.2.5 Resistance Exercise Session

For details of the resistance exercise session, please refer to section 3.5.

5.2.6 Interventions

For details of the interventions, please refer to section 3.6.

5.2.7 Body Composition

For details of the body composition assessment, please refer to section 3.7.

5.2.8 Intramuscular Temperature

Intramuscular temperature was measured using a re-usable sterile needle thermistor (MKA08050-A, Ellab A/S, Rodovre, Denmark) with data read via a thermocouple system (E-Val Flex, Ellab A/S, Rodovre, Denmark). The needle thermistors were sterilised using an autoclave prior to each use according to manufacturer guidelines. The site of insertion was identified as the mid-point of the vastus lateralis muscle of the right limb, between the superior border of the patella and the inguinal fold and was marked using a pen for consistency between assessments with the site of insertion sterilised using a topical antiseptic (Betadine, Purdue Products LP, CT, USA). To ensure consistency in the depth of insertion, skin thickness and adipose tissue was measured using callipers and a piece of medical tape was placed from the end of the needle at a distance which corresponded to 3 cm plus half of the skinfold thickness. The needle was then inserted into the vastus lateralis muscle until the tape contacted the skin surface, ensuring an intramuscular temperature of 3 cm. Once the reading had stabilised (approximately 2 seconds), the temperature was recorded. The needle was then manually

removed to a depth of 2 cm and the temperature recorded once the reading had stabilised. This process was repeated for a depth of 1 cm prior to the needle being removed. Previous research has used this method to determine the effect of water immersion on intramuscular temperature following resistance exercise (Mawhinney et al., 2017).

5.2.9 Maximal Voluntary Isometric Contraction

For procedures related to MVIC assessment, please refer to section 3.8.1.

5.2.10 Isometric Squat

For details of the isometric squat assessment, please refer to section 3.8.2.

5.2.11 Active Muscle Soreness

For details of the muscle soreness assessment, please refer to section 3.9.

5.2.12 Blood Sample Collection and Analysis

Venous blood was collected as outlined in section 3.10. The time of collection for all blood markers was based upon the recommended sampling points from chapter 1 (Table 5.2).

Table 5.2 List of blood markers and associated time points of collection.

Marker	Baseline	2 h	24 h
CK	✓		✓
hsCRP	✓		✓
IL-6	✓	✓	✓
IL-10	✓	✓	✓
MMP-9	✓	✓	✓

CK, creatine kinase; hsCRP, high-sensitivity C-Reactive protein; IL-6, interleukin-6; IL-10, interleukin-10; MMP-9, matrix metalloproteinase-9.

5.2.12.1 Creatine Kinase

For analysis of CK, please refer to section 3.10.1.

5.2.12.2 High Sensitivity C-Reactive Protein

For analysis of hsCRP, please refer to section 3.10.2.

5.2.12.3 Interleukin-6

For analysis of IL-6, please refer to section 3.10.3.

5.2.12.4 Interleukin-10

For analysis of IL-10, please refer to section 3.10.3.

5.2.12.5 Matrix Metalloproteinase-9

For analysis of MMP-9, please refer to section 3.10.4.

5.2.13 Statistical Analysis

Data were analysed by making probabilistic magnitude-based inferences as described in more detail in section 3.11. A paired samples t-test was performed to check differences between HWI and PAS groups in baseline characteristics with mechanistic inferences derived using a published spreadsheet (Hopkins, 2017a). To see the effect of the exercise bout for measures of acute physiological responses, data from both groups combined were checked for differences from baseline to each subsequent time point using a published spreadsheet (Hopkins, 2017b). The smallest worthwhile change was used to determine the effect of the independent variable on each dependent variable using a spreadsheet designed for analysis of a parallel groups trial. Comparisons were made between baseline and each subsequent time point (i.e. baseline-2 h, baseline-24 h) with differences compared between the HWI and PAS groups.

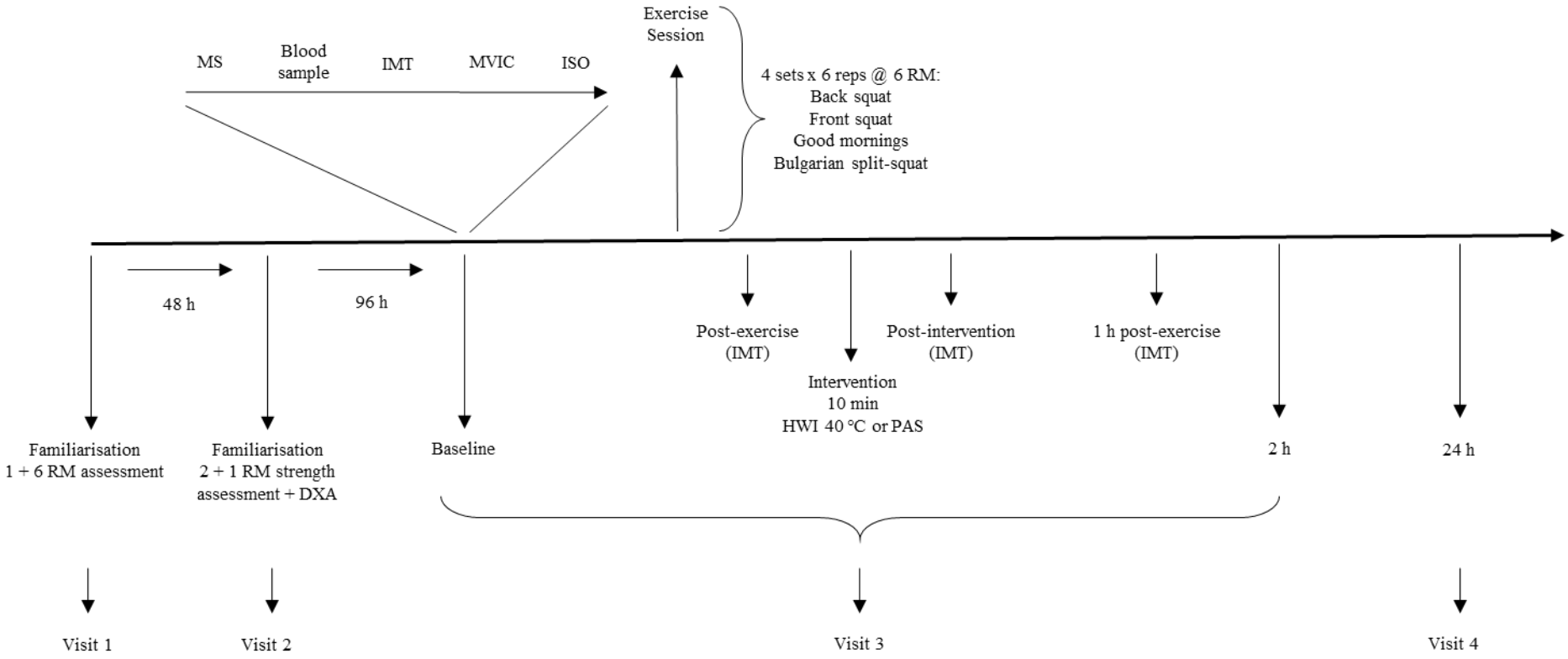

Figure 5.1 Schematic overview of the study timeline. MS, muscle soreness; IMT, intramuscular temperature; MVIC, maximal voluntary isometric contraction; ISO, isometric squat; RM, repetition maximum; DXA, dual-energy x-ray absorptiometry; HWI, hot water immersion; PAS, passive recovery

5.3 Results

5.3.1 Intramuscular Temperature

Intramuscular temperature at baseline was recorded at muscle depths of 3 cm (35.85 ± 0.76°C, PAS; 35.95 ± 0.67°C, HWI), 2 cm (34.81 ± 1.11°C, PAS; 35.06 ± 0.73°C, HWI) and 1 cm (33.17 ± 1.18°C, PAS; 33.52 ± 0.76°C, HWI). Immediately post-exercise, increases in intramuscular temperature for both groups were: *most likely* very large at 3 cm (1.7; ± 0.3°C), *most likely* large at 2 cm (1.5; ± 0.5°C), and *very likely* moderate at 1 cm (0.9; ± 0.8°C), with differences between groups *unclear* (3 cm 0.3; ± 0.5°C; 2 cm 0.5; ± 1.1°C; 1 cm 0.8 ± 1.8°C) (Figure 5.2). Following the intervention, combined group increases were: *most likely* moderate at 3 cm (0.8; ± 0.4°C), *very likely* moderate at 2 cm (0.9; ± 0.6°C), and *likely* moderate at 1 cm (0.7; ± 0.8°C). Combined group effects were *unclear* at 1 h post-exercise (3 cm 0.0; ± 0.4°C; 2 cm 0.0; ± 0.5°C; 1 cm 0.2; ± 0.6°C). The post-intervention increase was greater for HWI with a *most likely* large effect compared to PAS (3 cm 1.1; ± 0.4°C; 2 cm 1.7; ± 0.7°C; 1 cm 1.9; ± 0.9°C), which remained *very likely* moderately greater at 1 h post-exercise (3 cm 0.7; ± 0.6°C; 2 cm 0.9; ± 0.8°C). By 2 h post-exercise, intramuscular temperature had decreased from baseline with combined group effects: *most likely* moderate at 3 cm (−0.7; ± 0.4°C) and 2 cm (−0.9; ± 0.6°C), and *very likely* moderate at 1 cm (−0.8; ± 0.6°C). Differences between groups at 2 h post-exercise were *unclear* (3 cm 0.2; ± 0.8°C; 2 cm 0.4 ± 1.2°C; 1 cm 0.6; ± 1.2°C).

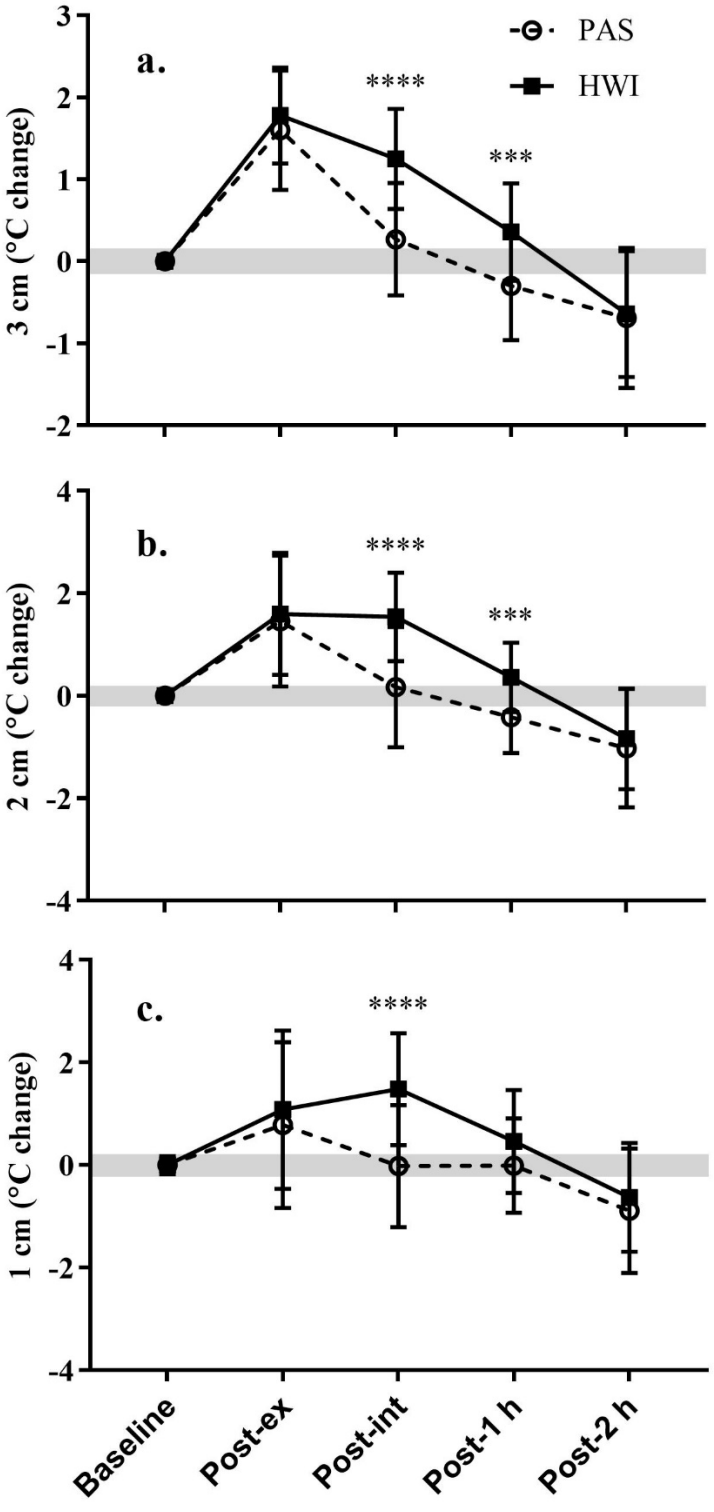

Figure 5.2 Raw change (°C) from baseline for intramuscular temperature at depths of (a) 3 cm, (b) 2 cm and (c) 1 cm. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between passive recovery (PAS) and hot water immersion (HWI) to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

5.3.2 Maximal Voluntary Isometric Contraction

Baseline values for MVIC force were 226 ± 39 N and 258 ± 99 N for PAS and HWI groups, respectively. Following the resistance exercise session, there was a *very likely* moderate decrease in peak force for both groups at 2 h (−16; ± 7.2%) and *likely* small decrease at 24 h (−7.2; ± 3.8%), although differences between groups were *unclear* (2 h −1.9; ± 19%; 24 h −1.2; ± 8.4%) (Figure 5.4).

5.3.3 Isometric Squat

At baseline, peak force was 2157 ± 573 N and 1952 ± 369 N for PAS and HWI groups, respectively. There was a *very likely* small decrease in peak force for both groups following the resistance exercise session at 2 h (−12; ± 8.2%) and 24 h (−14; ± 9.3%), although differences between groups were *unclear* (2 h −5.4; ± 18%; 24 h 8.1; ± 24%) (Figure 5.4).

Baseline values were reported for the following RFD time intervals: $RFD_{0\text{-}100}$ (718 ± 287 $N \cdot s^{-1}$, PAS; 653 ± 163 $N \cdot s^{-1}$, HWI) and $RFD_{100\text{-}200}$ (361 ± 177 $N \cdot s^{-1}$, PAS; 434 ± 134 $N \cdot s^{-1}$, HWI). At 2 h following the resistance exercise session, combined group changes in RFD were: *likely* moderate for $RFD_{0\text{-}100}$ (−23; ± 20%) and *unclear* for $RFD_{100\text{-}200}$ (−2.4; ± 22%), with differences between groups *unclear* ($RFD_{0\text{-}100}$ −6.0; ± 48%; $RFD_{100\text{-}200}$ −4.6; ± 45%). The pooled group changes in RFD at 24 h post-exercise were: *very likely* moderate for $RFD_{0\text{-}100}$ (−23; ± 13%) and *unclear* for $RFD_{100\text{-}200}$ (−0.5; ± 21%), with *unclear* differences between groups ($RFD_{0\text{-}100}$ 9.2; ± 39%; $RFD_{100\text{-}200}$ −18; ± 36%).

5.3.4 Active Muscle Soreness

At baseline, muscle soreness ratings were 5 ± 4% and 1 ± 1% for PAS and HWI groups, respectively. Following the resistance exercise session there was a *most likely* large increase for both groups in muscle soreness at 2 h post-exercise (6.0; x/÷ 2.0), and a *most likely* very large increase at 24 h post-exercise (14; x/÷ 1.7), with differences *unclear* between groups (2 h 2.6; x/÷ 3.9; 24 h 1.5; x/÷ 3.9) (Figure 5.4).

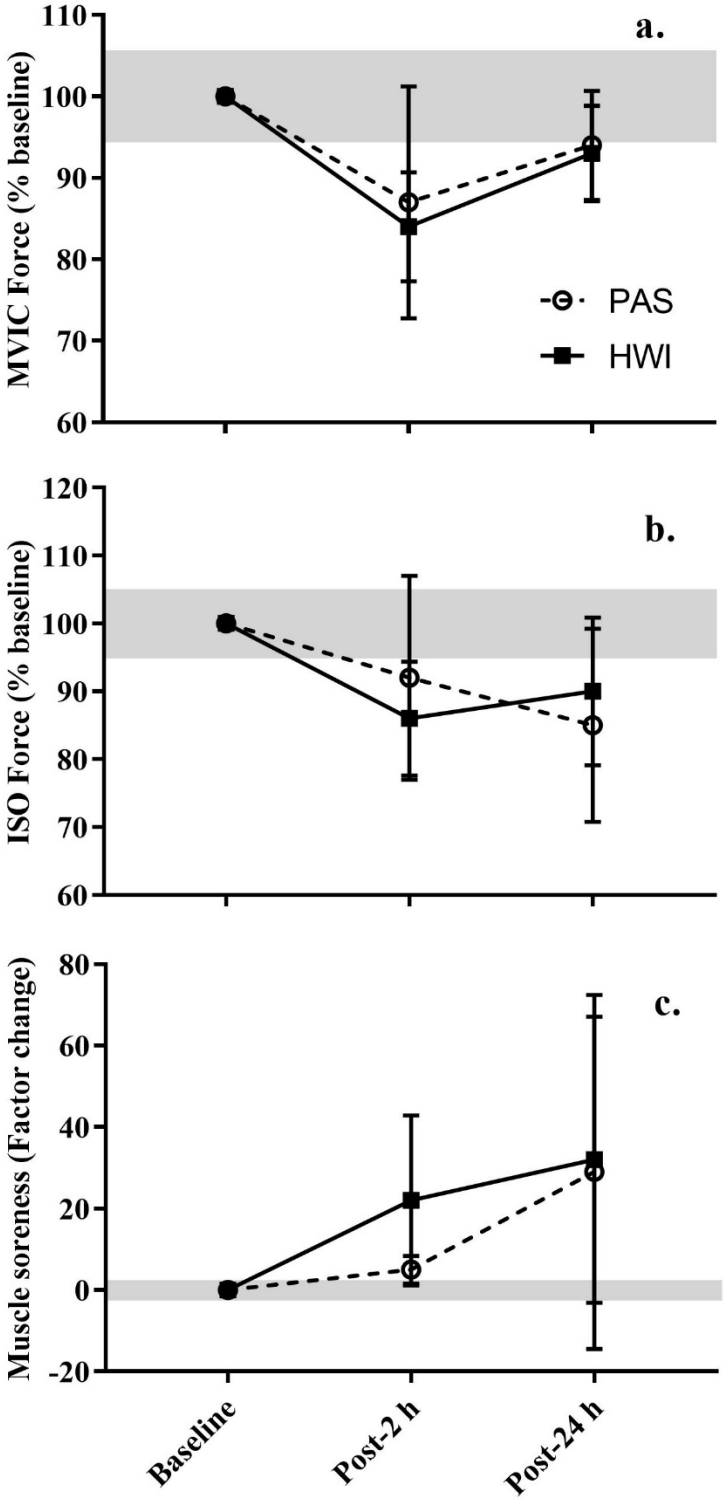

Figure 5.3 Change from baseline for measures of (a) maximal voluntary isometric contraction (MVIC), (b) isometric squat (ISO) and (c) muscle soreness. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between passive recovery (PAS) and hot water immersion (HWI) to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

5.3.5 Blood Analysis

Concentrations of hsCRP at baseline were 3.4 ± 6.3 mg·L^{-1} and 1.2 ± 1.0 mg·L^{-1} for PAS and HWI groups, respectively. Concentrations were *possibly* increased for both groups at 24 h post-exercise (1.2; x/÷ 1.3) with the increase in hsCRP *likely* smaller for HWI (−0.66; x/÷ 1.5) compared to PAS (Figure 5.5).

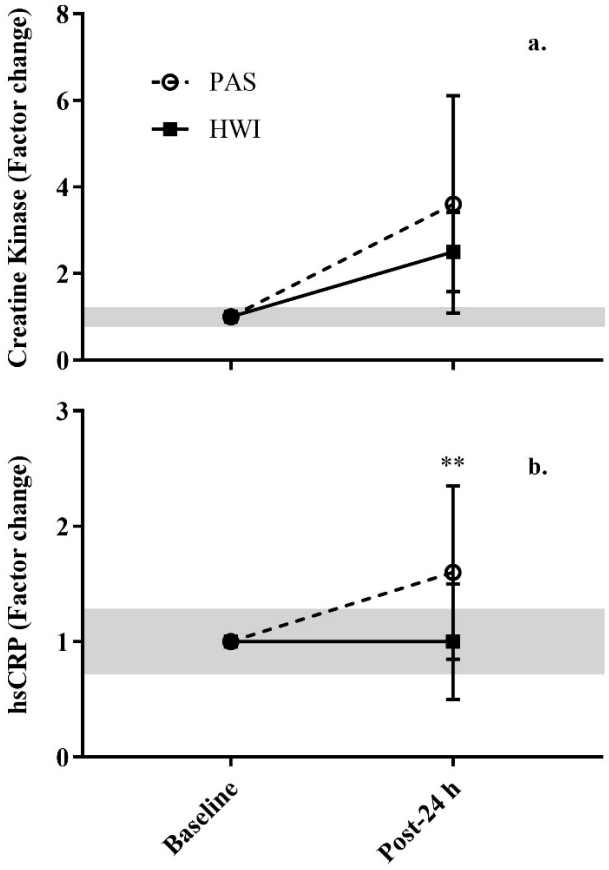

Figure 5.4 Change from baseline in (a) creatine kinase and (b) high sensitivity C-reactive protein (hsCRP) concentrations. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between passive recovery (PAS) and hot water immersion (HWI) to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

Baseline CK concentrations were 464 ± 407 U·L^{-1} and 686 ± 917 U·L^{-1} for PAS and HWI groups, respectively. Concentrations were *most likely* moderately elevated for both groups at 24 h post-exercise (2.5; x/÷ 1.3), with *unclear* differences between the groups (0.81; x/÷ 1.7) (Figure 5.5).

At baseline, concentrations of IL-6 were 1.3 ± 0.8 pg·mL^{-1} and 0.9 ± 0.5 pg·mL^{-1} for PAS and HWI groups, respectively. Following the resistance exercise session, concentrations were

most likely moderately increased for both groups at 2 h (1.7; x/÷ 1.3) and *unclear* at 24 h (1.4; x/÷ 1.6), with differences between groups *unclear* (2 h 0.79; x/÷ 1.7; 24 h 0.75; x/÷ 2.9) (Figure 5.6).

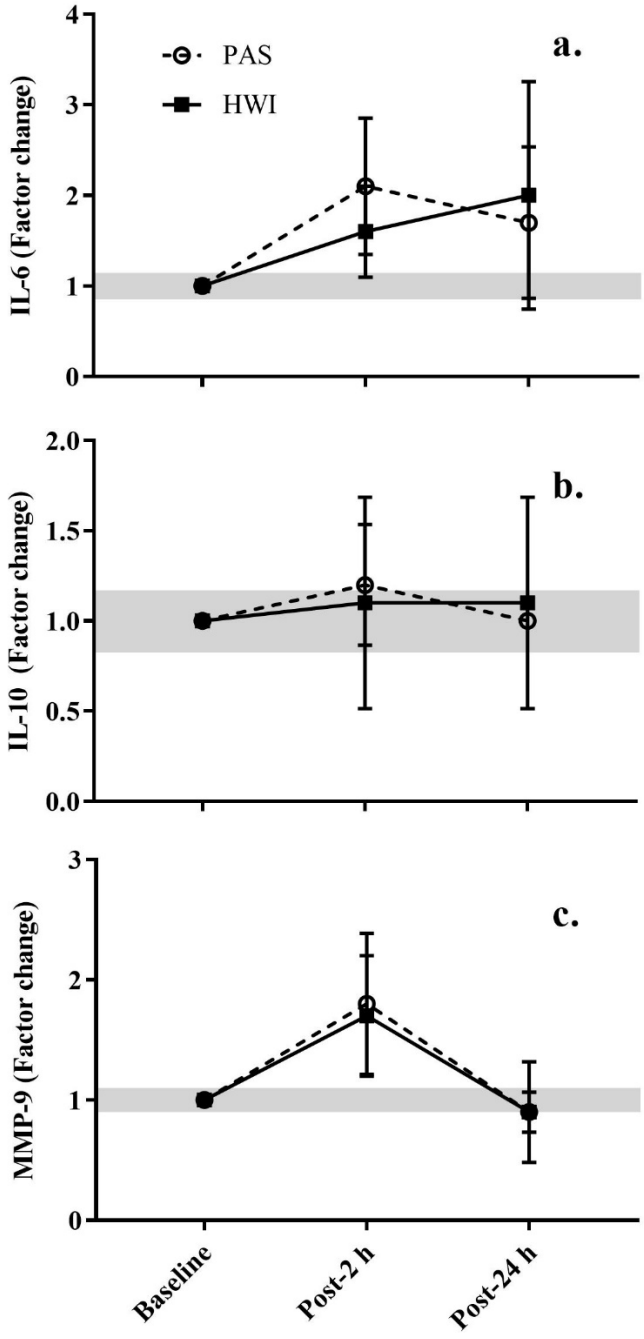

Figure 5.5 Change from baseline in (a) interleukin-6 (IL-6), (b) interleukin-10 (IL-10) and (c) matrix metalloproteinase-9 (MMP-9) concentrations. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between passive recovery (PAS) and hot water immersion (HWI) to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

Concentrations of IL-10 at baseline were 2.7 ± 2.9 pg·mL^{-1} and 3.8 ± 2.4 pg·mL^{-1} for PAS and HWI groups, respectively. Post-exercise changes in the concentration of IL-10 were *unclear* for both groups at 2 h (1.0; x/÷ 1.5) and 24 h (0.86; x/÷ 1.4), with *unclear* differences between groups (2 h 0.79; x/÷ 2.1; 24 h 0.9; x/÷ 1.9) (Figure 5.6).

Baseline concentrations for MMP-9 were 802 ± 378 ng·mL^{-1} and 644 ± 242 ng·mL^{-1} for PAS and HWI groups, respectively. There was a *most likely* moderate increase in MMP-9 concentration for both groups at 2 h post-exercise (1.7; x/÷ 1.2), before a *likely* small decrease at 24 h (0.83; x/÷ 1.2), with differences between groups *unclear* (2 h 0.86; x/÷ 1.4; 24 h 0.98; x/÷ 1.5) (Figure 5.6).

5.4 Discussion

The purpose of this study was to investigate the effect of HWI on acute physiological responses to resistance exercise. The results of this study indicate that HWI is a viable method of heat therapy that can maintain the rise in intramuscular temperature following resistance exercise. We also noted a suppressed response of hsCRP at 24 h following the exercise bout in the HWI group. Despite these results, there were no clear effects of HWI on measures of muscle function, muscle soreness or other blood markers of muscle cell disruption or inflammatory processes. Collectively, these findings suggest that (i) post-exercise increases in intramuscular temperature can be maintained through the superficial application of heat, (ii) maintaining elevated intramuscular temperature may manipulate inflammatory processes, and (iii) clear effects for recovery of muscle function and muscle soreness are independent of HWI-associated changes in intramuscular temperature. These results represent the first investigation into the acute physiological responses of a 'real-world' HWI protocol following resistance exercise, alongside the use of a trained cohort, applied exercise session and utilising good nutritional practice.

This is the first study to investigate the effects of HWI on intramuscular temperature during recovery from resistance exercise. After the post-exercise elevation in intramuscular temperature for both groups, the PAS group saw a decline following the intervention, whilst the HWI group maintained an elevated temperature up to 1 h post-exercise. Immediately following the intervention, HWI had the greatest effect on superficial tissue temperature, which is in line with the temperature gradient of skeletal muscle whereby shallow depths will be impacted the most by superficial temperature changes (Faulkner et al., 2012). This also explains why at 1 h post-exercise, there were no differences between groups at a 1 cm depth, whilst the intramuscular temperature at 3 cm and 2 cm continued to be elevated following HWI.

As previously hypothesised (Wilcock et al., 2006), the HWI-induced increase in tissue temperature would be expected to induce peripheral vasodilation and a subsequent increase in muscle blood flow. In the acute post-exercise period, a reduction in muscle blood flow, associated with cold water immersion, has typically been viewed as beneficial for exercise recovery due to reductions in inflammation, oedema and pain (Lee et al., 2005). This viewpoint has been challenged by recent research showing cold water immersion to exert no influence on muscle inflammatory or cellular stress responses (Peake et al., 2016). The hypothesised increase in muscle blood volume post-HWI in the present study was not seen alongside changes in most inflammatory markers or muscle soreness. These results build on previous evidence to suggest that changes in muscle blood volume associated with water immersion after resistance exercise, do not influence exercise recovery with any impact likely due to independent effects of changes in intramuscular temperature.

Perhaps the most striking finding of this study was that HWI completely blunted the increase in hsCRP that was seen following PAS at 24 h post-exercise. This is in line with the supposition that increases in muscle blood flow facilitate increased permeability of cellular, lymphatic and capillary vessels (Wilcock et al., 2006), leading to greater clearance rates of inflammatory markers in the blood. Given this, it is surprising that none of the other blood markers in this study displayed similarly enhanced rates of clearance following HWI, especially IL-6 which is a systemic precursor to hepatic hsCRP production (Donges et al., 2010). Additionally, clearance rates for hsCRP are reported to be constant suggesting that the blood concentration is derived solely from synthesis (Ramamoorthy, Nallasamy, Raghavendra Reddy, & Maruthappan, 2012). This study also reports IL-10, as a marker of anti-inflammatory processes, to be unaffected by HWI. There was also no change after the bout of resistance exercise, which provides further support for this finding in Chapter 4. Together, these are the first results to suggest that HWI may influence pro-inflammatory processes following resistance exercise, possibly by directly blunting hepatic hsCRP production.

Only two studies investigating HWI have collected inflammatory markers from the blood after exercise, both of which have shown no effect (Pournot et al., 2011; Vaile et al., 2008b), however neither analysed hsCRP as a proxy measure of acute-phase inflammation (Ramamoorthy et al., 2012). A mixture of findings related to post-exercise HWI and the appearance of intramuscular proteins in the blood currently exists with evidence of: a reduction (Vaile et al., 2008b), a rise (Viitasalo et al., 1995) and no effect (Pournot et al., 2011). The only study to find an enhanced clearance utilised multiple immersions for each day up to 72 h post-exercise (Vaile et al., 2008b). It is therefore reasonable to suggest that like others, the single bout of HWI in this study did not enhance the clearance of markers in the blood. Although speculative, perhaps more likely is that the HWI protocol used in the present

study elicited a direct effect on aspects of the inflammatory response, however the reasons why only hsCRP was blunted without clear effects on IL-6/IL-10/MMP-9 are yet to be determined. Research has shown both passive heat therapy (Kobayashi et al., 2005; Uehara et al., 2004; Yoshihara et al., 2013) and heat therapy in addition to a mechanical stimulus (Goto et al., 2003; Kakigi et al., 2011) to upregulate key anabolic signalling pathways and protein expression. The increased intramuscular temperature per se may therefore exert direct effects on cell signalling, impacting both inflammatory and anabolic pathways, although further research is required to confirm this.

Despite the manipulation of physiological responses that would in theory aid the recovery of muscle function, we found that the effect of HWI on MVIC, ISO peak force or RFD was unclear compared to PAS. These results may be partially attributed to a reduction in acute physiological responses seen here in comparison to those reported in Chapter 4 which would have reduced the likelihood for an effect of the intervention. For example, at 24 h post-exercise the reduction in MVIC was 13% in Chapter 4 and 7.2% in the present study. A possible cause could be differences in participant cohort, whereby those in Chapter 4 had a 1 RM of 1.4 x BM, whilst the participants in the present study demonstrated 1.8 x BM. However, strength is not the primary determinant of training status (Buckner et al., 2017) and participants were recruited from an identical inclusion/exclusion criteria. Another possible explanation is the addition in the present study of added nutritional control which included the provision of protein supplements in the acute post-exercise period. Previous research has demonstrated that consuming protein (in the form of branched chain amino acids) can attenuate reductions in muscle function in the post-exercise period in resistance trained individuals (Howatson et al., 2012). This may therefore explain the reduced response seen between Chapter 4 and the present study. However, best practice recommendations (Jager et al., 2017) would suggest individuals to consume protein in the post-exercise period and therefore in line with the applied nature of this study, the inclusion of the nutritional control enhanced ecological validity.

Previous research has typically produced mixed results related to the effect of post-exercise heat therapy on the recovery of muscle function, with those in support showing beneficial effects on all-out exercise performance (Cheng et al., 2017), strength (Vaile et al., 2008b) and power (Viitasalo et al., 1995), although the mechanisms are still undefined. Cheng et al. (2017) reported that mean power output was better maintained following endurance exercise when the upper limbs were heated (~38°C) compared to control (~33°C) or being cooled (~15°C). The authors attributed this to higher rates of glycogen resynthesis with heating. It is unlikely that measures of muscle function in the present study were limited by muscle glycogen stores and highlights the need to match post-exercise strategies to specific recovery

demands (Minett & Costello, 2015). Participants in the study by Cheng et al. (2017) were heated to an intramuscular temperature of ~38°C at a depth of 1.5 cm, which is greater than the post-intervention intramuscular temperature of 35.0 and 36.6°C reported in the present study for 1 and 2 cm, respectively. Despite differences in exercise modality, those that have reported beneficial effects of HWI, have used water temperatures of 37-38°C and durations of 14-20 min (Vaile et al., 2008b; Viitasalo et al., 1995). It is unlikely that these protocols would have elevated intramuscular temperatures to greater levels than the present study, therefore we speculate that the intramuscular temperatures we report would have been comparable to previous studies that have reported beneficial effects. This lends to the assertion that in this instance, recovery of muscle function during maximal strength tasks following resistance exercise is independent of short-term changes in intramuscular temperature and muscle blood volume associated with HWI.

The finding that HWI exerted unclear effects on ratings of muscle soreness is consistent with several reports (Kuligowski et al., 1998; Pournot et al., 2011; Vaile et al., 2008b). Studies investigating heat therapy that have demonstrated beneficial effects have been limited to those that included underwater massage alongside HWI (Viitasalo et al., 1995) or continuous (8 hour) low-level heat wrap therapy (Mayer et al., 2006) and would therefore be expected to elicit a markedly different response to HWI in the present study. Heat therapy has been suggested to reduce pain via an analgesic effect on nerves (Baker et al., 2001), although this theory has been proposed within the field of physiotherapy. Together, these results suggest that for those interested in applying HWI following a single bout of resistance exercise, it is unlikely to produce reductions in muscle soreness.

A number of points are worth considering when interpreting the results of this study. Firstly, a passive recovery arm was employed as the comparator to HWI. Although it is unlikely that athletic individuals will employ a sedentary post-exercise strategy, to understand the effect of HWI on acute physiological responses, it is recommended to compare with no recovery method (White & Caterini, 2017). Secondly, it is important to understand the implications of these findings in the context of the study. This study recruited trained individuals, employed an applied exercise modality, delivered a realistic HWI protocol and utilised good nutritional practice around the session to enhance the ecological validity of the findings. It is not possible to rule out that the influence of heat therapy on physiological responses may differ in other situations. For example, passive heat acclimation has been shown to improve muscle contractile properties which may have clinical relevance for individuals unable to exercise (Racinais et al., 2016). Given that this study has demonstrated HWI to impact upon inflammatory processes, and the known role of inflammation on repair and regeneration of skeletal muscle, the implications of regular HWI after training must be considered.

In summary, this is the first study to demonstrate HWI as a viable means of heat therapy that can increase intramuscular temperature. These findings also offer insights into the effect of HWI on acute physiological responses in a real-world environment. In the context of this study it appears that HWI is no more effective than passive recovery for the recovery of muscle function, soreness and some markers of muscle cell disruption and inflammation following resistance exercise. Nevertheless, it is reported that HWI impacted on aspects of the inflammatory response. Further research is required to investigate the application of HWI in other contexts including: following other exercise modalities, using different durations/temperatures of immersion, and when used chronically as part of a long-term training programme.

5.5 Perspectives

This chapter addressed the second and third aims of the thesis, to *'Characterise the hypothesised physiological mechanisms (intramuscular temperature) to a practical HWI protocol'*, and to *'Assess the effect of HWI on acute physiological responses and recovery following resistance exercise'*. Participants in the HWI group demonstrated an elevated intramuscular temperature for up to 1 h post-exercise compared to those in the PAS group. HWI blunted the response of hsCRP, a marker of the inflammatory response, although no effect was seen on measures of muscle function, muscle soreness or other blood markers. These results suggest that HWI can influence the physiology of post-exercise muscle temperature to manipulate aspects of the acute physiological response. This study builds on chapter 4 by incorporating the recommended measures and time points of assessment for acute physiological responses.

This is the first study to demonstrate that a real-world HWI protocol can maintain elevated intramuscular temperature following resistance exercise. This supports the purported mechanisms that underpin the use of HWI, as discussed in section 2.5.2 of this thesis, and provides the first empirical evidence for such a theory. The HWI protocol utilised in the present study employed a moderate water temperature (40°C) and duration of immersion (10 min) such that the protocol could realistically be implemented by applied sports scientists or even recreational exercisers within leisure facilities, enhancing the ecological validity of the findings.

The results of this study highlight that HWI is a strategy that can manipulate aspects of the acute physiological response, although the absence of an effect on muscle function or soreness suggests its efficacy may be limited in scenarios where the goal is exercise recovery. A potential limitation of this study is that assessments were only taken up to 24 h post-exercise and any effects that may have potentially been seen after this time point were not included

within the analysis. However, the findings of chapter 4 indicate that of the acute physiological responses assessed, the peak change would have occurred within 24 h (except for muscle soreness). It is also unlikely that for individuals engaged in regular resistance exercise a period of greater than 24 h without exercise would be afforded between sessions and therefore this study focussed on a period that would be relevant to those in the field, supporting the applied nature of this thesis.

Despite the limited influence of HWI on exercise recovery, the acute physiological responses that were manipulated (intramuscular temperature, inflammatory response) may be relevant within the context of adaptation to resistance exercise. To date, no studies have investigated the effect of regular HWI on adaptation to a resistance training programme or if the influence on acute physiological responses would be maintained following a training programme. Therefore, the following chapter of this thesis will address this gap in the literature.

6 Effect of Hot Water Immersion on Acute Physiological Responses and Training Adaptation Following a 10-week Resistance Training Programme

This chapter investigated the effect of HWI on acute physiological responses and training adaptation following a 10-week resistance training programme. The main findings demonstrated that HWI (i) augmented long-term gains in strength, (ii) had no effect on the post-training increase in lower body lean mass (iii) elicited an accelerated recovery of muscle function and soreness in the acute post-exercise period following training, and (iv) attenuated the increase of markers of inflammation and muscle cell disruption following training compared to PAS. Collectively, these findings suggest that at the end of a 10-week training programme, HWI manipulates acute physiological responses to hasten post-exercise recovery. This may have positively impacted an individual's ability to train in subsequent sessions, leading to an accumulated training stimulus that induced small but worthwhile improvements in strength.

6.1 Introduction

The previous chapter of this thesis demonstrated that HWI manipulates acute physiological responses (intramuscular temperature, inflammatory response) that may be relevant within the context of adaptation to resistance training. To build on these findings within the main theme of this thesis, the current chapter will investigate the effect of HWI on training adaptation and acute physiological responses following a resistance training programme. Additionally, the recommended measures and time points of assessment from Chapter 4 will be employed, whilst the same cohort, HWI protocol and resistance exercise session from Chapter 5 will be utilised.

As shown in Chapter 4, a single bout of resistance exercise stimulates a range of acute physiological responses which reduce muscle function and performance, supporting conventional wisdom (Howatson & Van Someren, 2008). As such, a variety of strategies have been developed to reduce these perturbations with the aim of enhancing exercise recovery. However, recent evidence suggests that acute physiological responses following resistance exercise play a key role in instigating beneficial adaptations following long-term training (Schoenfeld, 2012). Pertinent to this suggestion are reports that CWI attenuated long-term gains in muscle mass and strength which the authors attributed to a blunted activation of key proteins and satellite cells in the acute post-exercise period (Roberts et al., 2015). Therefore, an understanding of both the acute and chronic effects of recovery strategies are warranted to improve prescription for the purposes of optimising recovery and adaptation.

Due to its availability, HWI is a strategy commonly employed by both recreational exercisers and athletic populations (Versey, Halson, & Dawson, 2013). Despite reports of a beneficial influence on acute physiological responses (Mayer et al., 2006; Vaile et al., 2008b; Viitasalo et al., 1995), the results of Chapter 5 support the equivocal findings of others to show no effect of HWI after resistance exercise on muscle soreness, the appearance of intramuscular enzymes in the blood and the recovery of muscle function. However, it was found that HWI influenced aspects of the inflammatory response as well as maintaining a greater post-exercise intramuscular temperature, which are responses that are also implicated in adaptation to training. However, to date no published studies have investigated the effect of HWI on acute physiological responses following a training programme.

The HWI-induced maintenance of an elevated intramuscular temperature and muscle blood volume following resistance exercise (see Chapter 5), would in theory aid muscle protein synthesis due to its dependence upon an adequate blood supply (Fujita, Rasmussen, Cadenas, Grady, & Volpi, 2006). Additionally, in contrast to the evidence on CWI, heat therapy has been shown to upregulate key anabolic signalling pathways and protein expression following

both passive treatments (Kobayashi et al., 2005; Uehara et al., 2004; Yoshihara et al., 2013) and with the presence of a mechanical stimulus (Goto et al., 2003; Kakigi et al., 2011). This may be related to increased tissue temperatures promoting rates of enzymatic processes which has previously been shown to enhance glycogen resynthesis (Cheng et al., 2017). Although these studies provide a rationale for why HWI may complement resistance training, there has not been an investigation into the effect of regular HWI on changes in muscle mass and strength following a resistance training programme.

There is currently a need for studies to provide evidence into the effect of post-exercise HWI in a chronic setting in humans (McGorm et al., 2018; Méline et al., 2017). Given the growing use of heat therapy in recreationally active and athletic populations, further insights are required to improve scientific knowledge in this area for the purposes of optimising strategies to suit the specific recovery/adaptation goals of the individual (Minett & Costello, 2015). Therefore, the aim of the present study was twofold: (i) to examine the influence of regular HWI on training adaptation and (ii) to investigate the effect of HWI on a range of acute physiological responses following 10 weeks of resistance training in a trained cohort.

6.2 Methods

6.2.1 Participants

Sixteen strength trained males volunteered to take part in the study and were the same participants as described in Chapter 5 (Table 5.1). The study was granted ethical approval (application number: 1686) by the London Sports Institute Ethics Sub-Committee at Middlesex University.

6.2.2 Experimental Design

Using a between-subject design, participants were pair matched for baseline strength and body composition (body surface area to body mass ratio) and assigned to HWI or passive recovery (PAS) groups, as per Chapter 5 (Table 5.1). Participants attended the laboratory on 26 occasions (Figure 6.1). The first four visits of the study occurred as per section 5.2.2. Briefly, participants were familiarised with experimental procedures on visit 1 and 2 as well as performing 6 RM (visit 1) and 1 RM (visit 2) strength assessments and a body composition assessment (visit 2). Visit 3 formed the start of the experimental period and participants performed baseline assessments for muscle soreness, MVIC and ISO as well as providing a blood sample. Following baseline assessments, participants performed a resistance exercise session and then completed either the HWI or PAS interventions. The baseline assessments were repeated during visit 3 at 2 h post-exercise, before participants returned to the lab at 24 h post-exercise (visit 4) to repeat the baseline assessments again.

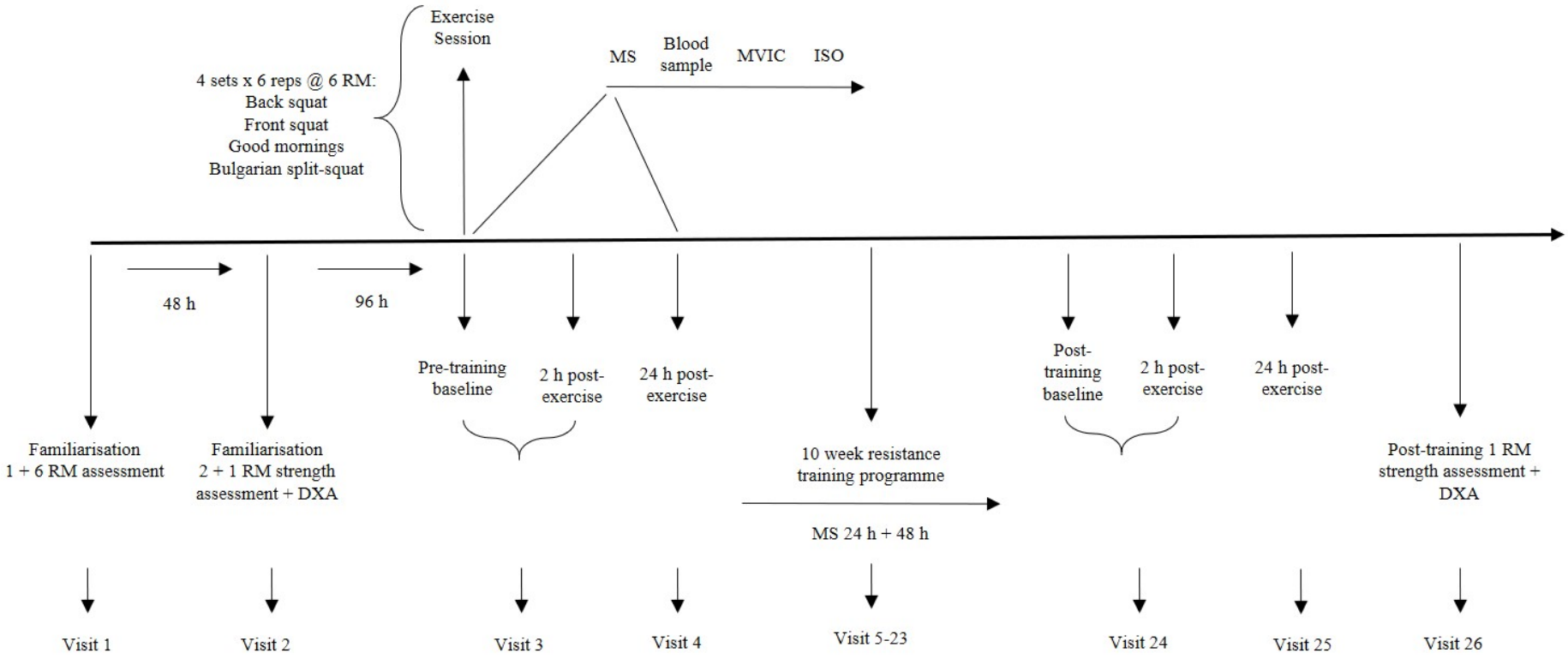

Figure 6.1 Schematic overview of the study timeline. MS, muscle soreness; MVIC, maximal voluntary isometric contraction; ISO, isometric squat; RM, repetition maximum; DXA, dual-energy x-ray absorptiometry; HWI, hot water immersion; PAS, passive recovery

During visits 5-23, participants performed a progressive resistance training programme (Table 6.1). Training was performed twice per week with a minimum of 48 h separating sessions (Figure 6.1). Muscle soreness was assessed at 24 h and 48 h following each training session. Participants replicated the experimental procedures of visit 3 (baseline, 2 h post-exercise) and 4 (24 h post-exercise) during visits 24 (baseline, 2 h post-exercise) and 25 (24 h post-exercise) for a comparison of the pre and post-training programme acute physiological responses. During visit 26, participants completed post-training programme strength and body composition assessments. Participants were required to avoid lower-body strength exercise external to the protocol and any therapeutic interventions throughout the study.

6.2.3 Dietary Control

Participants were asked to refrain from food consumption in the two hours prior to any testing procedures or training sessions throughout the study. Participants were required to avoid alcohol in the 24 h prior to any visit as well as nutritional supplements and non-steroidal anti-inflammatory drugs throughout the study. Aside from the control measures outlined below, participants were instructed to maintain their habitual dietary intake throughout the study.

As part of the aim to assess the adaptation to the training programme, participants were asked to record their nutritional intake in the 48 h prior to visit 2 and then to repeat this nutritional intake in the 48 h prior to visit 26. As per previous research (Roberts et al., 2015), standardised nutrition was provided around each training session to limit potential individual responses to the training. For each training session, participants were provided with a protein milk to consume 2 h prior to each training session and following completion of the HWI or PAS intervention, between which they were required to avoid any other food consumption. Additionally, participants were provided with a protein bar (20 g protein) to consume 2 h after each training session.

To aid in assessing the change in acute physiological responses following the resistance exercise session pre- and post-training programme, on the morning of visits 3 (pre-training programme baseline and 2 h post-exercise assessments) and 24 (post-training programme baseline and 2 h post-exercise assessments), participants were provided with a standardised meal (27 g porridge oats, 180 mL semi-skimmed milk, 200 g high protein yoghurt) to consume 2 h before arrival at the laboratory. Participants also consumed a ready to drink protein milk (30 g protein) following completion of the exercise session and after the 2 h post-exercise assessments, between which they were required to be fasted (Roberts et al., 2015). Participants were asked to record their nutritional intake in the period from 48 h prior to visit 3 until the completion of visit 4 (pre-training programme 24 h post-exercise assessment) and then to

repeat this nutritional intake in the period from 48 h prior to visit 24 until the completion of visit 25 (post-training programme 24 h post-exercise assessment).

6.2.4 6 Repetition Maximum

For details of the 6 RM assessment, please refer to section 3.4.1.

6.2.5 1 Repetition Maximum

For details of the 1 RM assessment, please refer to section 3.4.2.

6.2.6 Resistance Exercise Session

For details of the resistance exercise session, please refer to section 3.5.

For the post-training programme resistance exercise session, the load was determined from the 6 RM assessment during visit 23 (Table 6.1) to ensure a comparable intensity from pre- to post-training programme.

6.2.7 Resistance Training Programme

In each of the visits 5-23, participants completed a supervised lower body resistance training programme which utilised the four exercises from the resistance exercise session. Training sessions were performed twice per week with a minimum of 48 h separating sessions and a standardised warm-up was performed before each session. Initial training loads were set at a percentage of each participants 1 RM for each exercise which was predicted based upon the 6 RM assessment using a validated equation (Wathen, 1994). Resistance training was progressive, and the programme included periodic 6 RM assessments to reassign training loads. The intensity and volume of the sessions were selected based upon recommendations that loads of 80-95% 1 RM elicit maximal gains in strength (Peterson et al., 2005), and hypertrophy (Fry, 2004). The performance of at least 8-10 weekly sets per muscle group has also been suggested to be required to maximise increases in muscle strength (Peterson et al., 2005) and size (Schoenfeld et al., 2016) in trained individuals. Two minutes rest was afforded between sets and exercises, which has been recommended as a minimum for maximising gains in muscle size (Schoenfeld, Pope, et al., 2015). See Table 6.1 for further details.

6.2.8 Interventions

For details of the interventions, please refer to section 3.6.

6.2.9 Body Composition

For details of the body composition assessment, please refer to section 3.7.

6.2.10 Maximal Voluntary Isometric Contraction

For procedures related to MVIC assessment, please refer to section 3.8.1.

Table 6.1 Resistance training programme.

Exercise	(Visit number) Sets x repetitions @ % 1 RM
Back squat	(5, 7) 3 x 10 @ 75% (6) 3 x 6 @ 85% (9, 11) 4 x 8 @ 80% (10, 12) 4 x 6 @85% (14, 16) 4 x 8 @ 80% (15, 17) 4 x 5 @ 87% (19, 21) 4 x 8 @ 80% (20, 22) 4 x 4 @ 90% (8, 13, 18, 23) 6 RM assessment
Front squat	(5, 7) 3 x 10 @ 75% (6) 3 x 6 @ 85% (9, 11) 4 x 8 @ 80% (10, 12) 4 x 6 @85% (14, 16) 4 x 8 @ 80% (15, 17) 4 x 5 @ 87% (19, 21) 4 x 8 @ 80% (20, 22) 4 x 4 @ 90% (8, 13, 18, 23) 6 RM assessment
Good morning	(5, 7) 3 x 10 @ 75% (6) 3 x 6 @ 85% (9, 11) 3 x 8 @ 80% (10, 12) 3 x 6 @85% (14, 16) 3 x 8 @ 80% (15, 17) 3 x 5 @ 87% (19, 21) 4 x 8 @ 80% (20, 22) 4 x 4 @ 90% (8, 13, 18, 23) 6 RM assessment
Bulgarian split-squat	(5, 7) 3 x 10 @ 75% (6) 3 x 6 @ 85% (9, 11) 3 x 8 @ 80% (10, 12) 3 x 6 @85% (14, 16) 4 x 8 @ 80% (15, 17) 4 x 5 @ 87% (19, 21) 4 x 8 @ 80% (20, 22) 4 x 4 @ 90% (8, 13, 18, 23) 6 RM assessment

Exercises were performed in descending order. The eccentric phase of each exercises was performed in a controlled fashion lasting approximately two seconds, whilst the concentric phase was performed with maximal acceleration. Two minutes rest was afforded between sets and exercises, leading to a total training session duration of approximately 60 min. RM, repetition maximum.

6.2.11 Isometric Squat

For details of the isometric squat assessment, please refer to section 3.8.2.

6.2.12 Active Muscle Soreness

For details of the muscle soreness assessment, please refer to section 3.9.

6.2.13 Blood Sample Collection and Analysis

Venous blood was collected as outlined in section 3.10. The time of collection for all blood markers was based upon the recommended sampling points from Chapter 4 (Table 5.2).

6.2.13.1 Creatine Kinase

For analysis of CK, please refer to section 3.10.1.

6.2.13.2 High Sensitivity C-Reactive Protein

For analysis of hsCRP, please refer to section 3.10.2.

6.2.13.3 Interleukin-6

For analysis of IL-6, please refer to section 3.10.3.

6.2.13.4 Interleukin-10

For analysis of IL-10, please refer to section 3.10.3.

6.2.13.5 Matrix Metalloproteinase-9

For analysis of MMP-9, please refer to section 3.10.4.

6.2.14 Statistical Analysis

Data were analysed by making probabilistic magnitude-based inferences as described in more detail in section 3.11.

6.2.14.1 Training Adaptation

Analysis of changes in muscle mass and strength (1 RM and 6 RM) was performed using a spreadsheet designed for analysis of a parallel groups trial (Hopkins, 2006). Comparisons were made between baseline (visit 2) and post-training programme (visit 26) with differences compared between the HWI and PAS groups. Differences between HWI and PAS groups for pooled muscle soreness ratings throughout the training programme were assessed using a published spreadsheet (Hopkins, 2006). Comparisons were made between HWI and PAS groups for data that was pooled at 24 h and 48 h across the training programme (visits 5-23). Baseline values of acute physiological responses were analysed for differences from pre- to post-training programme using a published spreadsheet (Hopkins, 2006). Comparisons were made between the baseline of the pre-training programme resistance exercise session (visit 3) and the baseline of the post-training programme resistance exercise session (visit 24) with differences compared between the HWI and PAS groups.

6.2.14.2 Acute Physiological Responses

To assess differences in acute physiological responses between HWI and PAS groups following a single bout of resistance exercise at the end of the training programme (visit 24 and 25), data were analysed using a spreadsheet designed for analysis of a parallel groups trial (Hopkins, 2006). Comparisons were made between baseline and each subsequent time point (i.e. baseline-2 h, baseline-24 h) with differences compared between the HWI and PAS groups. To assess how these acute physiological responses differed from pre- (visit 3 and 4) to post-training programme (visit 24 and 25), a spreadsheet designed for analysis of a crossover trial was used (Hopkins, 2017b). Comparisons were made between the change from baseline to each subsequent time point (i.e. baseline-2 h, baseline-24 h) following the resistance exercise session that was conducted pre- and post-training programme for the HWI and PAS groups.

6.3 Results

6.3.1 Training Adaptation

6.3.1.1 Strength Assessment

Following the training programme, combined data for both groups demonstrated a *most likely* moderate increase in 6 RM strength for the back squat (17; ± 10 kg, PAS; 20; ± 11 kg, HWI) and front squat (17; ± 14 kg, PAS; 24; ± 15 kg, HWI), *most likely* very large increase for the good morning (33; ± 19 kg, PAS; 24; ± 9 kg, HWI) and *most likely* large increase for the Bulgarian split-squat (28; ± 13 kg, PAS; 33; ± 10 kg, HWI). When looking at the differences between groups, HWI has a *possibly* small beneficial effect on the change in 6 RM Bulgarian split-squat (7.7; ± 12%) compared to PAS. Effects between groups for back squat (2.2; ± 7.1%), front squat (3.5; ± 11%) and the good morning (−6.3; ± 11%) were *unclear*.

Maximal strength (1 RM) was *very likely* increased (small effect) after the training for the PAS (8 ± 8 kg) and HWI groups (17 ± 13 kg). The increase in 1 RM was *possibly* greater for HWI (5.8; ± 7.9%, small effect) compared to PAS (Figure 6.2).

6.3.1.2 Body Composition

Lower body lean mass *possibly* increased (small effect) after the training for the PAS (0.6; ± 0.5 kg) and HWI (1.1; ± 0.9 kg) groups, with differences between the groups *likely* trivial (1.0; ± 3.6%) (Figure 6.2). After training, the change in body mass was *very likely* trivial for both PAS (−1.3; ± 1.4 kg) and HWI (1.1; ± 1.7 kg), with differences between groups *likely* trivial (2.3; ± 2.4%). Differences from pre- to post-training for leg tissue % fat were *very likely* trivial for PAS (−0.6; ± 1.4%) and *likely* trivial for HWI (−0.6; ± 1.1%), with differences between groups *likely* trivial (5.3; ± 10%). The change in whole body tissue % fat pre-post training was *likely* trivial for both PAS (−1.0; ± 1.7%) and HWI (−0.6; ± 1.3%), with *likely* trivial differences between groups (−0.8; ± 12%).

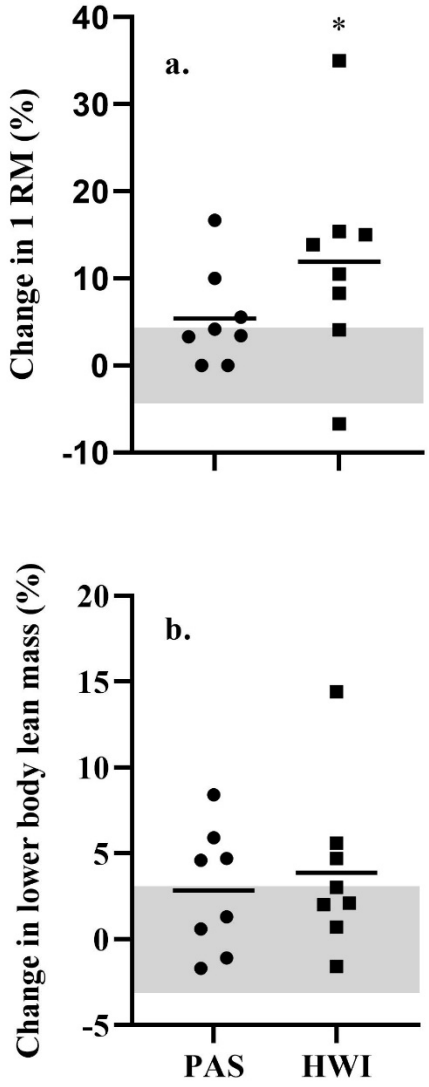

Figure 6.2 Change from baseline (%) in (a) 1 repetition maximum (RM) and (b) lower body lean mass for passive recovery (PAS) and hot water immersion (HWI) groups. The shaded area represents the smallest worthwhile change compared with the baseline value. Individual data are presented with mean change overlaid. The number of asterisks (*) indicate the likelihood for the changes between PAS and HWI to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

6.3.1.3 Active Muscle Soreness

Muscle soreness ratings pooled from across the training period were reported at 24 h (23 ± 10%, PAS; 21 ± 14%, HWI) and 48 h post-training (20 ± 12%, PAS; 12 ± 10%, HWI). Effects were *unclear* between groups at 24 h (0.67; x/÷ 2.2), while HWI *likely* reduced muscle soreness ratings at 48 h post-training (0.60; x/÷ 1.9, small effect) compared to PAS (Figure 6.3).

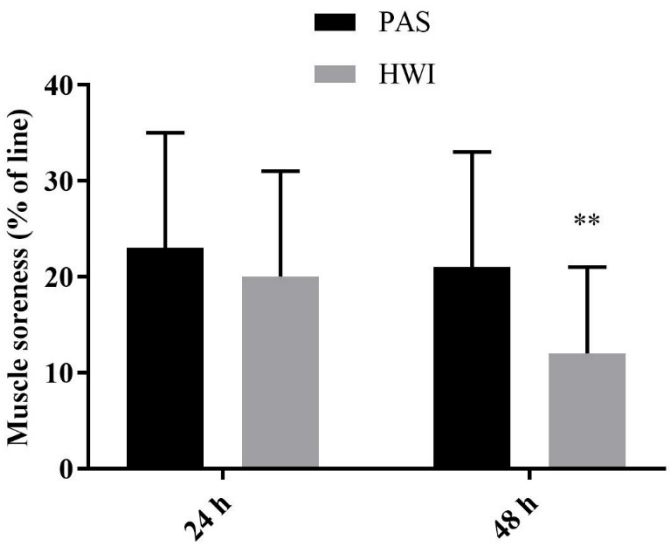

Figure 6.3 Pooled ratings of muscle soreness (% of line) at 24 h and 48 h following each training session for the passive recovery (PAS) and hot water immersion (HWI) groups. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between passive recovery (PAS) and hot water immersion (HWI) to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes

6.3.1.4 Baseline Values of Acute Physiological Responses

The change in baseline values for MVIC force before and after the training programme was *likely* trivial for both PAS (0.9; ± 3.5%) and HWI (−0.3; ± 7.9%), with differences between groups *unclear* (2.2; ± 8.6%) (Table 6.2).

Following the training programme, the change in baseline isometric squat peak force was *unclear* for both PAS (−1.2; ± 9.1%) and HWI (8.6; ± 15%), with differences between groups *unclear* (9.9; ± 17%).

Baseline $RFD_{0\text{-}100}$ was *very likely* slower from pre- to post-training programme for PAS (−24; ± 11%, moderate effect), whilst there were *unclear* differences for HWI (−3.1; ± 17%). The slower $RFD_{0\text{-}100}$ in the PAS group was a *likely* greater change than the HWI group (28; ± 27%, moderate effect) (Table 6.2). Baseline $RFD_{100\text{-}200}$ was *very likely* faster for PAS after the training porgramme (40; ± 23%, small effect), whereas the difference was *unclear* for HWI (11; ± 21%). The faster $RFD_{100\text{-}200}$ in the PAS group was a *likely* greater change than the HWI group (−20; ± 24%, small effect) (Table 6.2).

Muscle soreness ratings at baseline *likely* increased for PAS (1.7; x/÷ 1.6, small effect) and *very likely* increased for HWI (2.6; x/÷ 1.7, moderate effect) from pre- to post-training programme, with differences between groups *unclear* (1.6; x/÷ 2.0).

Changes in the concentration of hsCRP at baseline were *unclear* from pre- to post-training programme for both PAS (0.84; x/÷ 1.6) and HWI (0.79; x/÷ 2.1), with differences between groups *unclear* (0.8; x/÷ 2.1).

Baseline CK concentrations were *very likely* decreased in PAS (0.56; x/÷ 1.3, moderate effect) from pre- to post-training programme, while the difference was *unclear* for HWI (1.1; x/÷ 2.2). The change in PAS was *likely* different compared to the HWI group (2.1; x/÷ 2.1, moderate effect).

Baseline concentrations of IL-6 were *very likely* reduced from pre- to post-training programme in PAS (0.51; x/÷ 1.6, moderate effect), while the difference in HWI was *unclear* (0.86; x/÷ 1.6). The change in PAS was *possibly* greater compared to HWI (1.7; x/÷ 2.1, moderate effect).

Differences in the concentration of IL-10 at baseline were *unclear* from pre- to post-training programme for both PAS (1.4; x/÷ 1.7) and HWI (0.69; x/÷ 2.2), with differences between groups also *unclear* (0.51; x/÷ 2.4).

Changes in the baseline concentrations of MMP-9 from pre- to post-training programme were *unclear* for both PAS (0.86; x/÷ 1.4) and HWI (0.80; x/÷ 1.4), with differences between groups *unclear* (0.86; x/÷ 1.6).

Table 6.2 Baseline values for a range of variables pre- and post-training programme for the passive recovery (PAS) and hot water immersion (HWI) groups.

	PAS		HWI	
	Pre	Post	Pre	Post
MVIC (N)	226 ± 39	226 ± 22	258 ± 99	252 ± 74
ISO_{PF} (N)	2157 ± 573	2160 ± 707	1952 ± 369	2198 ± 666
$RFD_{0\text{-}100}$ ($N \cdot s^{-1}$)[a]	718 ± 287	551 ± 233*	653 ± 163	645 ± 202
$RFD_{100\text{-}200}$ ($N \cdot s^{-1}$)[a]	361 ± 177	464 ± 133*	434 ± 134	494 ± 206
MS (% of line)	5 ± 4	12 ± 12*	1 ± 1	4 ± 3*
hsCRP ($mg \cdot L^{-1}$)	1.2 ± 1.1	0.8 ± 0.6	1.2 ± 1.0	0.8 ± 1.0
Creatine kinase ($U \cdot L^{-1}$)[a]	464 ± 407	230 ± 174*	686 ± 917	755 ± 986
Interleukin-6 ($pg \cdot mL^{-1}$)[a]	1.3 ± 0.8	0.7 ± 0.5*	0.9 ± 0.5	0.9 ± 1.2
Interleukin-10 ($pg \cdot mL^{-1}$)	2.7 ± 2.9	5.8 ± 7.4	3.8 ± 2.4	3.6 ± 3.6
MMP-9 ($ng \cdot mL^{-1}$)	802 ± 378	675 ± 312	644 ± 242	565 ± 324

Data are presented as mean ± SD. PAS, passive recovery; HWI, hot water immersion; MVIC, maximal voluntary isometric contraction; ISO_{PF}, isometric squat peak force; $RFD_{0\text{-}100}$, rate of force development 0-100 ms; $RFD_{100\text{-}200}$, rate of force development 100-200 ms; MS, muscle soreness; hsCRP, high-sensitivity C-Reactive protein; MMP-9, matrix metalloproteinase-9. * clear effect versus pre, [a] clear difference in pre-post change between PAS and HWI.

6.3.2 Acute Physiological Responses

Post-training programme baseline values for measures of acute physiological responses are presented in Table 6.2.

6.3.2.1 Maximal Voluntary Isometric Contraction

Following the post-training programme resistance exercise session, there was a *likely* decrease in force at 2 h for both PAS (−12; ± 13%, moderate effect) and HWI (−12; ± 7.0%, small effect), although effects were *unclear* at 24 h for PAS (−7.6; ± 15%) and *likely* trivial for HWI (−3.1; ± 4.3%). Differences between groups were *unclear* at 2 h (0.5; ± 15%) and 24 h (5.0; ± 18%). Comparing the change from baseline to each time point from pre- to post-training programme, effects at 2 h (3.2; ± 25%) and 24 h (−1.6; ± 14%) were *unclear* for PAS. HWI *possibly* reduced the post-exercise decrease in force at 2 h (5.7; ± 8.6%) and 24 h (5.1; ± 8.8%) following training compared to pre-training programme (Figure 6.4).

6.3.2.2 Isometric Squat

After the post-training programme resistance exercise session, there was a *possible* decrease in force at 2 h for PAS (−5.7; ± 12%) and *likely* decrease for HWI (−13.1; ± 9.0%, small effect). At 24 h there was a *possible* decrease for PAS (−4.9; ± 8.8%), while the difference for HWI was *unclear* (−8.5; ± 14%). Differences between groups were *unclear* at 2 h (−7.9; ± 14%) and 24 h (−3.7; ± 16%). Comparing the change from baseline to each time point from pre- to post-training programme, effects at 2 h (4.3; ± 14%) were *unclear* for PAS, while there was a *likely* reduced (small effect) post-exercise decrease in force at 24 h (15; ± 23%) following the training programme compared to pre-training. Effects at 2 h (9.8; ± 29%) and 24 h (8.8; ± 35%) were *unclear* from pre- to post-training programme for HWI (Figure 6.4).

Following the post-training programme resistance exercise session, changes in $RFD_{0\text{-}100}$ were *unclear* at 2 h for PAS (22; ± 37%), while there was a *most* likely decrease RFD for HWI (−24; ± 8.2%, moderate effect). At 24 h, changes were *likely* trivial for PAS (4.9; ± 12%), while there was a *likely* decrease in RFD for HWI (−15; ± 13%, small effect). The reduction in $RFD_{0\text{-}100}$ at 2 h was *very likely* greater for HWI (−36; ± 21%, moderate effect) and *likely* greater at 24 h (−19; ± 14%, small effect) compared with PAS. Comparing the change from baseline to each time point from pre- to post-training programme, for PAS, effects at 2 h were *unclear* (50; ± 100%), while there was a *very likely* moderate increase in RFD showing greater recovery at 24 h (41; ±36%). For HWI, effects at 2 h (2.9; ± 41%) and 24 h (4.6; ± 19%) were *unclear*.

After the post-training programme resistance exercise session, changes in $RFD_{100\text{-}200}$ were *unclear* at 2 h for both PAS (−22; ± 36%) and HWI (−6.2; ± 29%). At 24 h, $RFD_{100\text{-}200}$ was *very likely* reduced for PAS (−24; ± 14%, moderate effect), while effects for HWI were

unclear (1.5; ± 20%). Differences between groups were *unclear* at 2 h (20; ± 62%), while at 24 h the recovery of $RFD_{100-200}$ was *very likely* greater for HWI compared to PAS (34; ± 33%, moderate effect). Comparing the change from baseline to each time point from pre- to post-training programme, for PAS, effects were *unclear* at 2 h (−22; ± 55%) and 24 h (−31; ± 36%). For HWI, effects were also *unclear* at 2 h (−3.6; ± 45%) and 24 h (12; ± 56%).

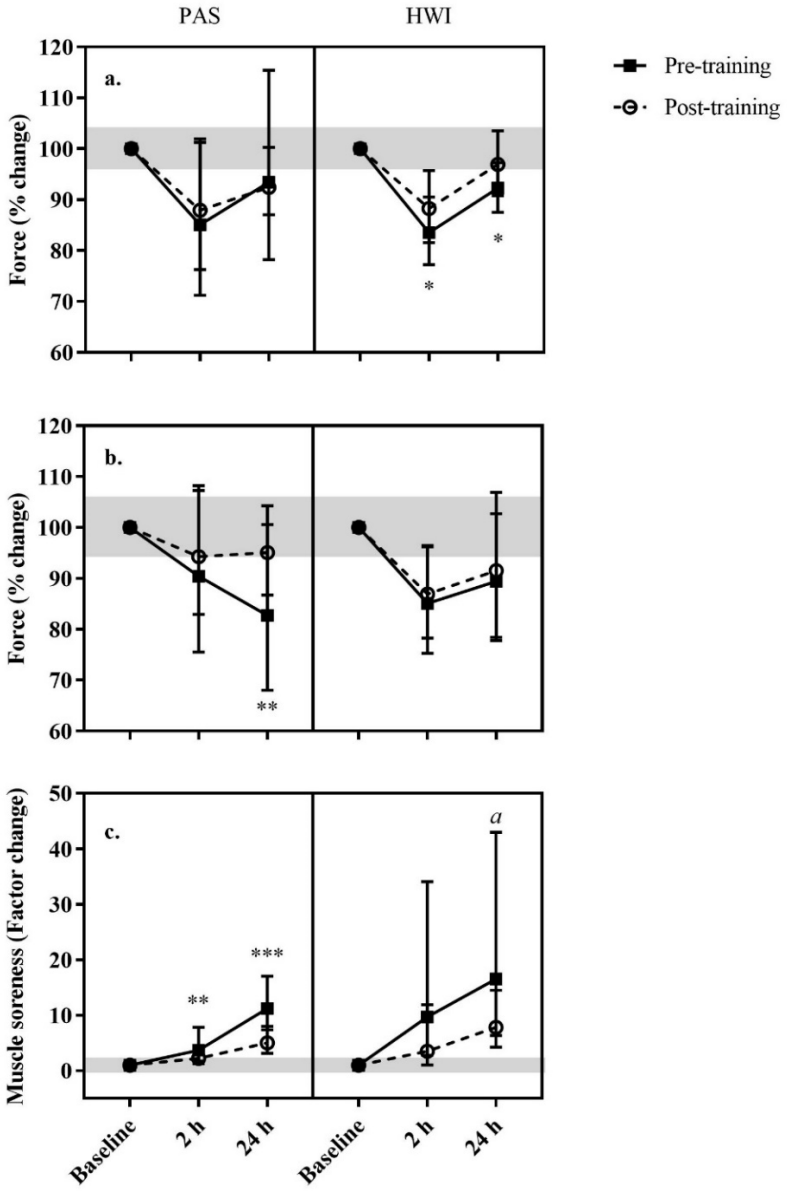

Figure 6.4 Change from baseline for both pre-training and post-training for measures of (a) maximal voluntary isometric contraction (MVIC), (b) isometric squat (ISO) and (c) muscle soreness for the passive recovery (PAS) and hot water immersion (HWI) groups. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes from pre- to post-training to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes. *a* refers to clear differences between the PAS and HWI groups in the post-training responses

6.3.2.3 Active Muscle Soreness

Following the post-training programme resistance exercise session, changes in ratings of muscle soreness were *very likely* increased at 2 h for both PAS (2.2; x/÷ 1.8, small effect) and HWI (3.5; x/÷ 3.4, moderate effect). At 24 h, muscle soreness ratings were *most likely* increased for both PAS (5.0; x/÷ 1.6, moderate effect) and HWI (7.8; x/÷ 1.9, large effect). Differences between groups were *unclear* at 2 h (1.6; x/÷ 3.7), while the increase in muscle soreness was *possibly* less at 24 h for HWI compared to PAS (0.6; x/÷ 2.2, small effect). Comparing the change from baseline to each time point from pre- to post-training programme, for PAS, ratings of muscle soreness were *likely* reduced at 2h (0.6; x/÷ 1.8, small effect) and *very likely* reduced at 24 h post-exercise (0.45; x/÷ 1.6, moderate effect) following the training programme. Effects at 2 h (0.36; x/÷ 3.9) and 24 h post-exercise (0.47; x/÷ 3.4) were *unclear* from pre to post training programme for the HWI group (Figure 6.4).

6.3.2.4 Blood Analysis

After the post-training programme resistance exercise session, changes in the concentration of hsCRP were *likely* increased at 24 h for PAS (1.4; x/÷ 1.4, small effect) whereas differences were *likely* trivial for HWI (1.1; x/÷ 1.2). The change in hsCRP was *possibly* smaller at 24 h for HWI compared to PAS (0.78; x/÷ 1.4, small effect). Change in the concentration of hsCRP from baseline to 24 h from pre- to post-training programme was *unclear* for both PAS (1.02; x/÷ 1.4) and HWI (1.08; x/÷ 1.4) (Figure 6.5).

Following the post-training programme resistance exercise session, CK concentrations at 24 h were *most likely* increased for PAS (2.4; x/÷ 1.2, large effect) and *likely* increased for HWI (1.5; x/÷ 1.3, small effect). The increase in CK concentration was *likely* smaller for HWI compared to PAS (0.62; x/÷ 1.8, small effect). The increase in CK concentration from baseline to 24 h from pre- to post-training programme was *likely* reduced for both PAS (0.65; x/÷ 1.8, small effect) and HWI (0.67; x/÷ 1.5, small effect) following training (Figure 6.5).

After the post-training programme resistance exercise session, there were *very likely* increases in IL-6 concentration at 2 h for PAS (2.6; x/÷ 1.8, large effect) and *most likely* increases for HWI (2.6; x/÷ 1.5, moderate effect). At 24 h, increased IL-6 concentrations were *very likely* for PAS (1.2; x/÷ 1.7, moderate effect), with effects *unclear* for HWI (1.1; x/÷ 1.4). Differences between groups were *unclear* at 2 h (1.0; x/÷ 2.0), while the increase in IL-6 concentration at 24 h was greater for PAS compared to HWI (0.55; x/÷ 1.8, moderate effect). Comparing the change from baseline to each time point from pre- to post-training programme, for PAS, there was a *likely* increase in IL-6 concentration at 2 h post-exercise (1.4; x/÷ 1.5, small effect) following the training programme compared with pre-training, while effects at 24 h (1.4; x/÷ 1.9) were *unclear*. For the HWI group, there was a *very likely* increase in the

concentration of IL-6 at 2 h post-exercise (1.4; x/÷ 1.2, small effect) from pre- to post-training programme, while effects at 24 h (0.73; x/÷ 1.7) were *unclear* (Figure 6.6).

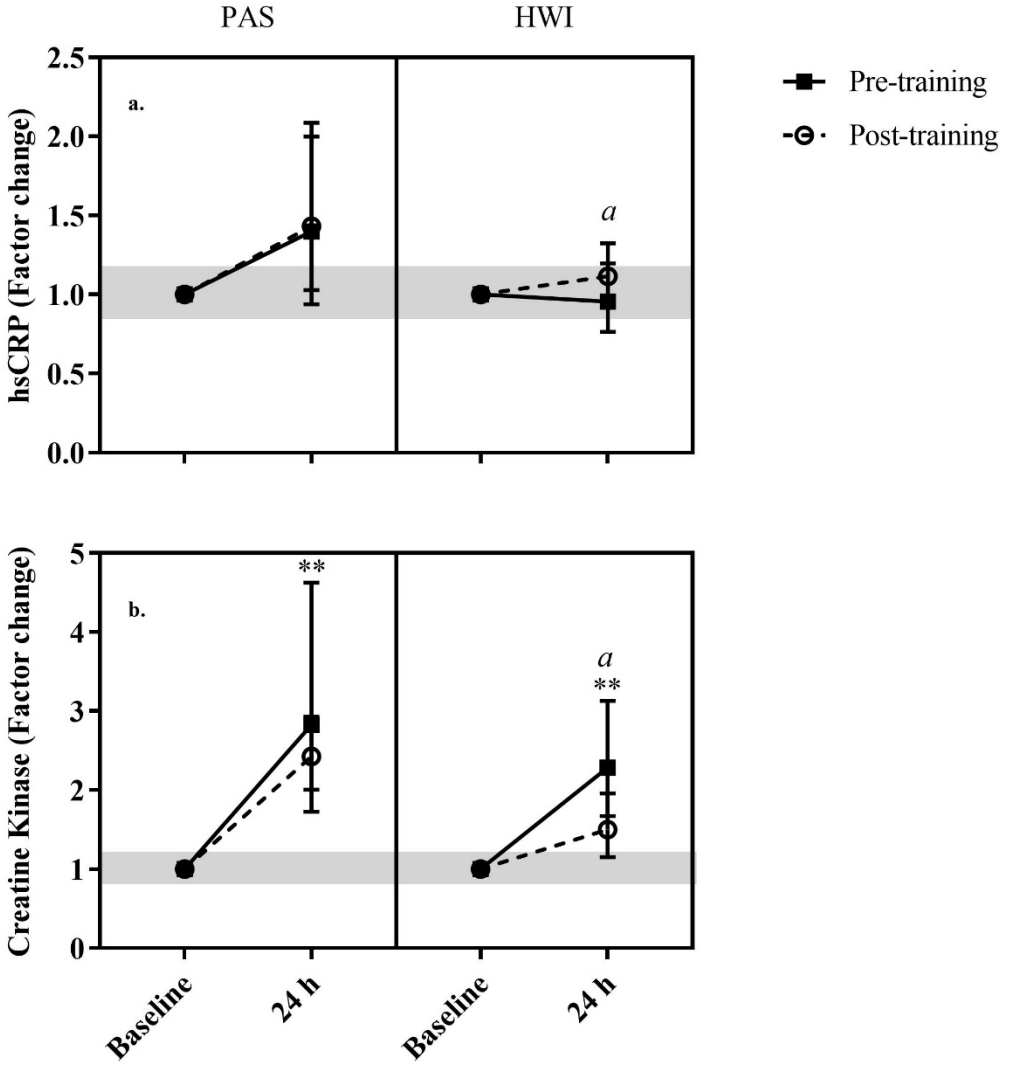

Figure 6.5 Change from baseline for both pre-training and post-training for concentrations of (a) creatine kinase and (b) high sensitivity C-reactive protein (hsCRP) for the passive recovery (PAS) and hot water immersion (HWI) groups. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between PAS and HWI to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes. *a* refers to clear differences between the PAS and HWI groups in the post-training responses

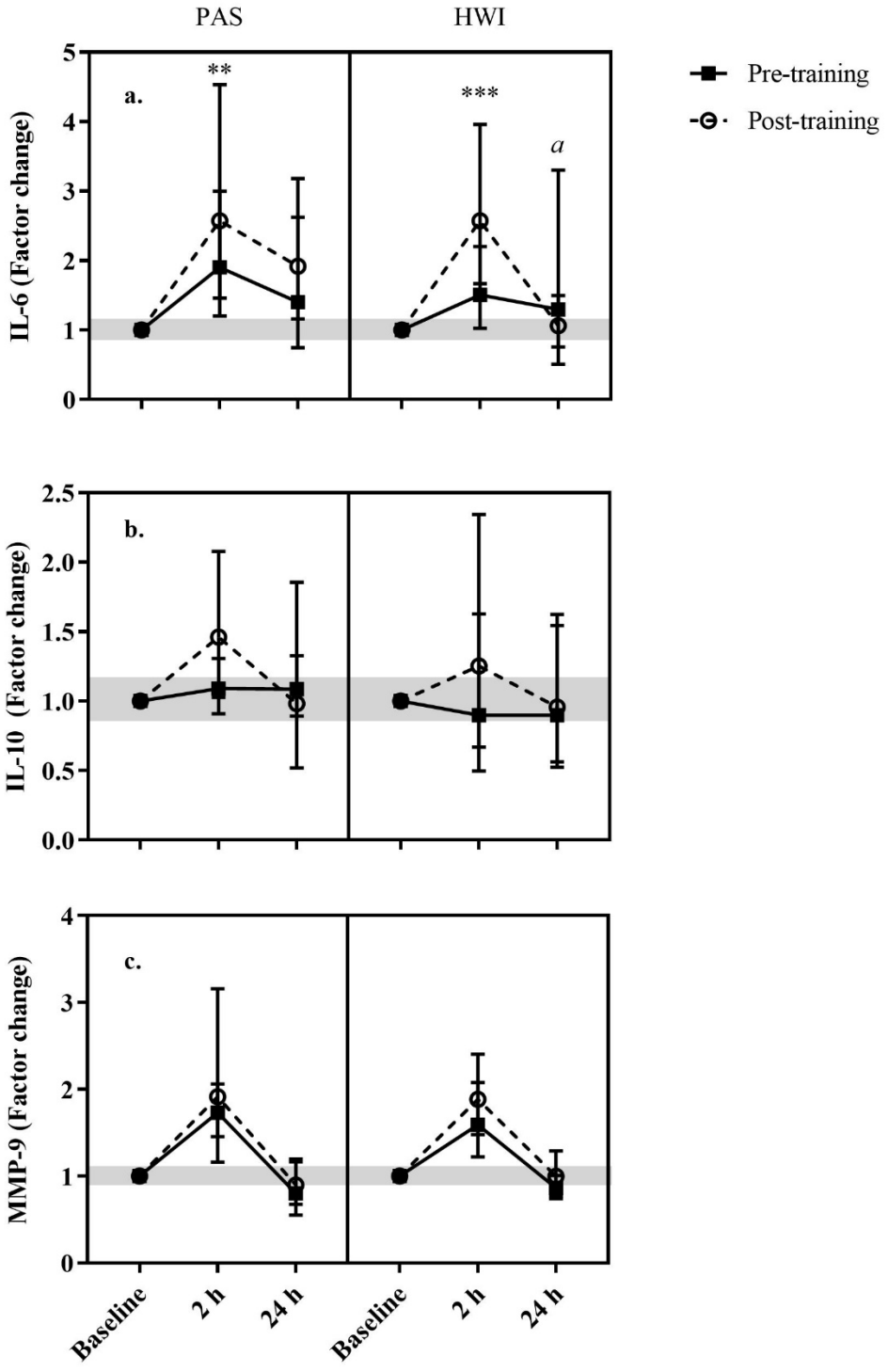

Figure 6.6 Change from baseline for both pre-training and post training for concentrations of (a) interleukin-6 (IL-6), (b) interleukin-10 (IL-10) and (c) matrix metalloproteinase-9 (MMP-9) for the passive recovery (PAS) and hot water immersion (HWI) groups. The shaded area represents the smallest worthwhile change compared with the baseline value. The error bars represent 90% confidence intervals. The number of asterisks (*) indicate the likelihood for the changes between PAS and HWI to be substantial, with * referring to possible changes, ** to likely, *** to very likely and **** to most likely changes. *a* refers to clear differences between the PAS and HWI groups in the post-training responses

Following the post-training programme resistance exercise session, IL-10 concentrations were *likely* increased at 2 h for PAS (1.5; x/÷ 1.4, small effect), while effects were *unclear* for HWI (1.3; x/÷ 1.9). At 24 h, effects were *unclear* for both PAS (1.0; x/÷ 1.9) and HWI (1.0; x/÷ 1.7). Differences between groups were *unclear* at 2 h (0.86; x/÷ 2.0) and 24 h (0.86; x/÷ 2.2). Comparing the change from baseline to each time point from pre to post training programme, changes in IL-10 concentrations at 2 h (1.3; x/÷ 1.8, PAS; 1.7; x/÷ 2.1, HWI) and 24 h post-exercise (0.80; x/÷ 2.2, PAS; 1.4; x/÷ 2.7, HWI) were *unclear* (Figure 6.6).

After the post-training programme resistance exercise session, concentrations of MMP-9 were *very likely* increased at 2 h for PAS (1.9; x/÷ 1.6, moderate effect) and *most likely* increased for HWI (1.9; x/÷ 1.3, moderate effect). At 24 h, effects were *unclear* for both PAS (0.90; x/÷ 1.3) and HWI (1.0; x/÷ 1.3). Differences between groups were *unclear* at 2 h (0.98; x/÷ 1.7) and 24 h (1.0: x/÷ 1.4). Comparing the change from baseline to each time point from pre- to post-training, change in the concentration of MMP-9 at 2 h was *unclear* for both PAS (1.1; x/÷ 1.8) and HWI (1.1; x/÷ 1.3) from pre- to post-training programme. At 24 h post-exercise, differences from pre- to post-training programme were *unclear* for both groups (1.0; x/÷ 1.7, PAS; 1.0; x/÷ 1.3, HWI) (Figure 6.6).

6.4 Discussion

The purpose of this study was to investigate the effect of HWI on training adaptation and acute physiological responses following 10 weeks of resistance training. The key findings related to training adaptation were that chronic post-training HWI (i) augmented long-term gains in 1 RM, (ii) had no effect on the post-training programme increase in lower body lean mass and (iii) reduced muscle soreness ratings at 48 h post-training throughout the programme compared to PAS. Additionally, (iv) PAS saw reductions in baseline CK and IL-6 compared to HWI, while there were no differences between groups for other measures. The key findings related to acute physiological responses were that chronic post-training HWI (i) attenuated the increase in muscle soreness as well as markers of inflammation and muscle cell disruption in the acute post-exercise period following the training programme compared to PAS, and (ii) enabled an enhanced recovery of MVIC and $RFD_{100-200}$ from pre- to post-training programme. Additionally, (iii) PAS saw accelerated recovery of ISO peak force and RFD_{0-100}, as well as reductions in muscle soreness ratings from pre- to post-training programme. Collectively, these findings suggest that at the end of a 10-week training programme, HWI manipulates acute physiological responses to hasten aspects of post-exercise recovery. This may have positively impacted an individual's ability to train in subsequent sessions, leading to an accumulated training stimulus that induced small but worthwhile improvements in strength. Considering the current study employed a trained cohort, an applied resistance training

programme and good nutritional practice, the findings present evidence that HWI may be of interest to athletic populations looking to gain a competitive advantage in the 'real world'.

6.4.1 Training Adaptation

This study is the first to investigate the impact of regular HWI on adaptations to an applied resistance training programme. The finding that HWI enhanced strength development is consistent with one of the few long-term human studies in the area. Goto (2007) showed that 10 weeks of heat stress combined with low-intensity exercise in the non-dominant arm, increases maximal isometric force to a greater extent than the dominant arm, that only performed exercise in the absence of heat stress. Individual responses presented here also show that in spite of both a high and low responder in the HWI group, the majority of individuals responded favourably in comparison to the PAS group. This is despite a similar average volume load performed per session throughout the training programme by both groups for the back squat (3339 kg, PAS; 3371 kg, HWI), with no differences seen between groups for any of the exercises. Clearly further work is required to strengthen the weight of evidence, however early indications are that regular HWI may positively influence strength adaptations following a resistance training programme.

Despite HWI benefits for strength, both the HWI and PAS groups increased lower body lean mass to a similar extent. Interpreting the individual traces provides further confirmation with the likeness of responses between the HWI and PAS groups. This supports the findings of (Stadnyk et al., 2018) but contrasts those of Goto (2007) who, in addition to increased maximal isometric force, noted greater hypertrophy of the biceps brachii with heat stress plus exercise. Given the training status of the participants in the present study, it is likely that the participants exhibited diminishing opportunity for adaptation which is congruent with the study by Goto (2007), where the participant with the highest cross-sectional area of the biceps brachii showed no change. The Goto (2007) study measured muscle size via computed tomography scans to get a cross-sectional image of the muscle. Computed tomography allows the assessment of individual muscle groups and may be more sensitive to detect changes compared to the DXA scan in the present study that produces a global measure of lower body lean mass. Additionally, differences in exercise modality may have contributed, whereby upper body models elicit greater increases in hypertrophy compared to those of the lower body (Abe, DeHoyos, Pollock, & Garzarella, 2000), and so the potential to see impact within this study may have been reduced. Another consideration is the timing of heat application as the present study and the paper by Stadnyk et al. (2018), both of which found no benefit of heating on hypertrophy, applied the heating protocol post-exercise and during plus post-exercise, respectively. This contrasts the findings of Goto (2007) who found heating before and during the exercise session to have a positive effect on hypertrophy. This may suggest the timing of

heat application to be a key determinant of the adaptive response for hypertrophy. As such, it is unlikely that the augmented strength with HWI in the present study is attributable to increases in muscle size. This is consistent with those that have challenged the viewpoint that an increase in muscle size will produce an increase in strength (Buckner et al., 2018), and could be due to changes in the neuromuscular system, for which resistance training is a potent stimulus (Deschenes & Kraemer, 2002). This supports the supposition that a greater training stimulus was possibly afforded with HWI in this study that led to the long-term benefits to strength. Further research investigating the impact of regular HWI across a range of exercise modalities and cohorts is required to further elucidate its potential benefits to increase muscle size with training.

Throughout the training programme, the HWI group displayed a reduction in the ratings of muscle soreness at 48 h post-exercise compared to the PAS group, which is a novel finding. Given that the second resistance training session of the week typically occurred 48 h following the first, it can be assumed that participants in the HWI group were performing this session with less feelings of muscle soreness than their counterparts in the PAS group. Although the exact mechanisms are yet to be defined, muscle soreness typically occurs alongside other acute physiological responses that may produce negative consequences in relation to strength, power and altered recruitment patterns (Cheung et al., 2003). Therefore, increased feelings of muscle soreness may be associated with a sub-optimal training intensity. This supports the viewpoint that HWI reduced feelings of muscle soreness at 48 h post-exercise throughout the training period, allowing individuals to perform the second session of the week with a greater intensity to support strength development.

Comparison of baseline values of acute physiological responses from pre to post training revealed there was no change in functional measures, while muscle soreness increased for both groups and the PAS group saw reductions in resting concentrations of CK and IL-6, with no change for other blood markers. When comparing the baseline values for these measures, it can be expected that resistance training will elicit: an increased force generating capability (Vincent & Vincent, 1997a), higher resting levels of CK (Mougios, 2007) and MMP-9 (Urso et al., 2009), lower resting levels of hsCRP and no difference on IL-6 (Donges et al., 2010). Although the participants in the current study engaged in a period of resistance training, they were already deemed ‘trained’ prior to the start of the programme and as such would be expected to have already gained any beneficial adaptations in relation to baseline values as part of their previous training. It is therefore surprising that the PAS group saw a decrease in CK and IL-6 after the programme which may be attributed to the variability in these measures across a 10-week period. This is supported by the findings from Chapter 4 whereby CK and IL-6 showed a typical error of 28% and 114%, respectively during a non-exercise control

week. The marked post-exercise response of the measures is what separates them as useful measures due to the signal:noise ratio and is a reminder that caution should be applied when comparing changes in these measures at rest. The increased baseline muscle soreness for both groups could possibly be related to inherent feelings of soreness associated with the programme which had accumulated by the end of the 10 weeks, especially given that identical control measures prior to each baseline session were instilled in the present study. While the reduction in $RFD_{0\text{-}100}$ and increased $RFD_{100\text{-}200}$ in the PAS group may have cancelled each other out to produce a similar peak force during the isometric squat. Together, these results suggest that HWI does not influence adaptations in relation to baseline values of muscle function, soreness or blood markers of inflammation and muscle cell disruption.

With regards to the nutritional control used in this study, protein supplementation has been shown to augment increases in muscle size and strength following resistance training (Cermak, Res, de Groot, Saris, & van Loon, 2012). To maximise the post-exercise stimulus, it is important to ensure adequate protein intake, without which might elicit suboptimal adaptations. Therefore, to control for differences in protein intake between individuals, this study included protein supplementation around each training session, as used by (Roberts et al., 2015). This approach, which would enhance the ecological validity of the study, would also reduce the window for an effect of HWI. Despite this, several positive findings were noted which highlights the potential application of this strategy in the real-world.

6.4.2 Acute Physiological Responses

We also assessed acute physiological responses both pre- and post-training to present the first insights into how these are impacted following training with regular HWI. There were no differences between groups for measures of muscle function in the acute post-exercise period at the end of the training programme. Studies investigating the effect of HWI on the recovery of muscle function have also produced equivocal findings (Pournot et al., 2011; Vaile et al., 2008b; Viitasalo et al., 1995), which is in line with the data at the start of the training programme (see Chapter 5). However, the recovery of MVIC was improved from pre- to post-training for the HWI group with the response for the PAS group being unchanged. The finding that HWI improved the recovery of force at the end of 10 weeks training is perhaps surprising. In line with the reduction in muscle soreness across the programme however, this supports the suggestion that participants in the HWI group were benefitting from an accelerated recovery. This may have allowed a better quality of session when a second training session was performed near the first, which proved advantageous for strength development.

There was no difference in the recovery of ISO peak force between groups at the end of the training programme. Additionally, there was an enhanced recovery of $RFD_{0\text{-}100}$ in the PAS

group compared to HWI, while the HWI had an enhanced recovery of $RFD_{100-200}$ compared to PAS. The finding that both groups improved the RFD in relation to one another at different time intervals explains that there was no difference in the recovery of ISO peak force. Differences between the groups for the different RFD time intervals may represent differences in ISO technique.

Ratings of muscle soreness were less at 24 h following the resistance exercise session at the end of the training programme for the HWI group compared to the PAS group, complimenting the finding of reduced muscle soreness for the HWI group across the training programme. This is despite the PAS group reporting less muscle soreness following the programme compared to the resistance exercise session pre-training. Baseline values were increased following training, possibly arising due to residual soreness from the programme, which may have artificially reduced the rise in soreness for the PAS group. Previous research has reported equivocal findings regarding the relationship between HWI and muscle soreness (Kuligowski et al., 1998; Pournot et al., 2011; Viitasalo et al., 1995), however we provide novel evidence that HWI may be a useful tool to reduce muscle soreness as part of a training programme. This further supports the assertion that participants in the HWI group were benefitting from an enhanced recovery throughout the training programme that afforded higher quality in the subsequent training session compared to individuals in the PAS group.

Following on from the novel finding in Chapter 5 that HWI blunts the post-exercise increase in hsCRP, the results of the present study follow this up to demonstrate that this response is maintained following 10 weeks of training. Further to this, in the present study HWI reduced the increased concentration of IL-6 at 24 h post-exercise compared to PAS. IL-6 is known to be a systemic precursor to hepatic hsCRP production (Donges et al., 2010) and therefore their congruent reduction is understandable. Post-training, both groups saw increased concentrations of IL-6 at 2 h post-exercise compared with pre-training, while the concentration of IL-10 increased in the PAS group compared with baseline. This may be attributable to the increased absolute workload following the training, which supports other reports of an enhanced cytokine response when the same relative workload is used from pre to post-training (Izquierdo et al., 2009). However, given the known relationship between IL-6 and hsCRP, HWI may have manipulated aspects of the inflammatory response such that concentrations of these markers were reduced by 24 h post-exercise compared with PAS. As suggested in the previous chapter, reductions of these markers in the circulation may be caused by an accelerated clearance due to HWI-induced increases in blood flow. The inflammatory response is a dynamic sequence of events that must be co-ordinated to support efficient tissue regeneration (Chazaud, 2016). Although speculative, it may be possible that following the training programme, HWI accelerated inflammatory processes through its impact on

increasing tissue blood flow, which could have contributed to the reductions in muscle soreness (Cheung et al., 2003). During the training programme this may have meant that the inflammatory response was beneficially accelerated with HWI so that by the time of the second session of the week, individuals were ready to receive a further training stimulus, thus benefitting strength development.

Following the training programme, there was a reduction in the post-exercise increase in CK concentrations for both groups. As would be expected, the reduction in post-exercise CK concentrations after a period of training demonstrates an adaptation congruent with the repeated bout effect (Howatson et al., 2007), even in trained lifters. A novel finding of the present study is there was a smaller post-exercise increase in CK for the HWI group compared to PAS after the training programme, which contrasts the findings of chapter 5. As with inflammatory processes, it may have been that HWI facilitated greater efficiency with the acute physiological responses throughout the programme, driven by elevated post-exercise blood flow, and that these responses only became apparent at the end of a period of training.

Several points are worth bearing in mind when interpreting these findings. Consideration must be given to the potential influence of placebo effects in this study. Previous reports have demonstrated that benefits of cold water immersion, including the recovery of strength, are not greater than the placebo effect (Broatch et al., 2014). However, inflammatory processes were manipulated in a manner that would be deemed beneficial for exercise recovery at the end of the training programme and it is unlikely that these would have been influenced by placebo effects. Additionally, a non-intervention control group is recommended to fully elucidate the impact of HWI (White & Caterini, 2017). It would therefore be prudent for future studies to investigate potential placebo effects associated with HWI. It is worth noting that the impact of HWI over a long-term training period in this study was small, but importantly beneficial. Given that trained individuals were recruited, an applied resistance training programme was employed, a realistic HWI protocol was delivered and good nutritional practice was utilised around the sessions to enhance the ecological validity of the findings, the small benefit may be of relevance to athletic populations striving for small enhancements in performance. In other contexts which allow greater opportunity for adaptation, for example in patients undertaking rehabilitation or elderly populations, HWI may provide a means of heat therapy to improve skeletal muscle function (Racinais et al., 2016) possibly leading to long-term gains in muscle size and strength which is an area for future research. The final point to be made is that taking everything into account, there appears very little evidence in this study to suggest any harmful effect of HWI. Given that it may in fact elicit some benefits, alongside positive perceptual responses, practitioners may be inclined to recommend trialling this strategy as a safe approach with athletes.

In summary, this is the first study to demonstrate HWI as a viable post-exercise strategy to enhance strength adaptations to an applied resistance training programme. We also offer insights into possible mechanistic contributors to this finding which include enhanced recovery of muscle function and soreness in the acute period after training, as well as manipulations to blood markers which may indicate a more efficient inflammatory response. These effects may have contributed to strength development by accelerating recovery and allowing individuals to train with greater intensity when a second training session was performed near the first. These results suggest that for athletic populations, HWI may be an adjunct to resistance training to augment recovery when sessions are performed in close proximity to offer additional small, but worthwhile, enhancements in strength.

6.5 Perspectives

This study addressed the final two aims of the thesis, to *'Investigate the influence of regular HWI on training adaptation following 10 weeks of resistance training'*, and to *'Examine the effect of HWI on acute physiological responses following a 10-week resistance training programme'*. Following the training, participants in the HWI group demonstrated a greater increase in 1 RM strength, although there was no effect of the increase in lower body lean mass. The HWI group reported less muscle soreness at 48 h post-training across the programme. After an acute bout of resistance exercise at the end of the training programme, the HWI group showed an accelerated recovery of muscle function and soreness as well as attenuations in the increase of markers of inflammation and muscle cell disruption compared to the PAS group. The results of this study suggest that HWI manipulates acute physiological responses to hasten post-exercise recovery at the end of a 10-week training programme. This may have positively impacted an individual's ability to train in subsequent sessions, leading to an accumulated training stimulus that induced small but worthwhile improvements in strength. This study builds on Chapter 4 and 5 by incorporating the recommended measures and time points of assessment for acute physiological responses as well as comparing these responses after a resistance training programme.

This is the first study to investigate the regular use of a practical HWI protocol throughout a progressive resistance training programme. These findings therefore provide novel evidence that HWI may support strength development during resistance training. This supports the mechanistic work from animal models and those investigating other methods of heat therapy, as discussed in section 2.5.4 of this thesis, and provides the first empirical evidence to support its use in a real-world environment. The use of a trained cohort, an applied resistance training programme and good nutritional practice, further enhance the ecological validity of the findings.

The results of this study also provide novel insights into the effect of HWI on acute physiological responses after a period of resistance training. The findings highlight that HWI continued to exert an effect at the end of the training programme, supporting its use as a training aid to accelerate recovery and allow for a greater intensity to be performed in subsequent sessions, which may have been advantageous for strength development. Additionally, the accelerated clearance of inflammatory and muscle cell disruption markers may suggest that HWI facilitated greater efficiency with the acute physiological responses throughout the programme, driven by elevated post-exercise blood flow, (as shown in Chapter 5) and that these responses only became apparent at the end of a period of training. These results suggest that for athletic populations, HWI may be an adjunct to resistance training to augment recovery when sessions are performed in close proximity to offer additional small, but worthwhile, enhancements in strength without compromising necessary acute physiological responses. This extends the application of HWI by providing recommendations for its regular use alongside training.

Despite a largely positive impact of HWI, interpretation of the findings must account for potential influences of the placebo effect. Given that the studies of this thesis are providing novel insights, others have recommended the comparison to a non-intervention control group to fully elucidate the impact of HWI (White & Caterini, 2017), which provides rationale for the current experimental design. However, as there are reports that placebo effects are associated with water immersion strategies (Broatch et al., 2014), future investigations should look to clarify potential placebo effects associated with HWI. In the meantime, because belief effects may be powerfully ergogenic (Halson & Martin, 2013) and this thesis does not report any negative consequences of HWI, applied physiologists and athletes may be interested to employ HWI as a strategy to support adaptations as part of a resistance training programme.

7 General Discussion

7.1 Experimental Chapter Synopsis

A variety of strategies exist with the goal of accelerating recovery from training and/or competition to improve subsequent performance by manipulating acute physiological responses to exercise. Recent evidence has suggested that altering these responses in pursuit of recovery aims may come at the detriment of adaptive processes, if used regularly as part of a training programme (Michailidis et al., 2013; Roberts et al., 2015; Trappe et al., 2002). These strategies have therefore been categorised within the concept of hormesis and are suggested to either dampen or enhance acute physiological responses to optimise the exercise stimulus (Peake, Markworth, et al., 2015). HWI is one such strategy that has received attention with regards to exercise recovery, likely due to its hypothesised ability to elevate muscle temperature and blood flow (Wilcock et al., 2006). These responses may be favourable for promoting exercise recovery, although supporting evidence is equivocal (Kuligowski et al., 1998; Pournot et al., 2011; Vaile et al., 2008a, 2008b; Viitasalo et al., 1995). The experimental chapters in this thesis aimed to build on the current body of evidence by investigating the influence of HWI on acute physiological responses and the subsequent impact on recovery from and adaptation to resistance exercise.

The first experimental chapter of this thesis (Chapter 4) was designed to identify sensitive measures to quantify acute physiological responses to resistance exercise as well as characterise the magnitude of change and time course of this response. The key aspects of acute physiological responses to resistance exercise are well accepted in the literature (Howatson & Van Someren, 2008). However, current understanding as to the usefulness of these measures is scarce. A measure's usefulness is underpinned by the relationship between the typical error (reliability), the magnitude of change post-exercise compared with the typical error (signal:noise) and the smallest change in the measure that is of importance to practitioners (Pyne et al., 2004). As a result, this study investigated a range of acute physiological response measures. The reliability was characterised across a time scale that is reflective of that in which the measure is to be used, as well as employing a control condition void of an exercise stimulus with the same participants to characterise the magnitude of change and time course of the response. The findings were that several of the measures investigated (MVIC, 20 m sprint, CMJ_{PF}, $RFD_{100\text{-}200}$, muscle soreness, limb girth, DALDA Part B, MMP-9, IL-6, CK, hsCRP and ascorbyl free radical) were sensitive measures to detect change in response to resistance exercise, with a signal-to-noise ratio of >1.5. Using these measures, clear effects were reported with aspects of the acute physiological response apparent as soon as 2 h, through to 96 h post-exercise. Relevant recommended measures and sampling points were then used throughout the thesis in subsequent experimental chapters.

The second investigation (Chapter 5) aimed to investigate the effect of HWI on acute physiological responses following resistance exercise. Despite the hypothesised effects, no studies have investigated the influence of HWI on intramuscular temperature. Of the research investigating the relationship between HWI and recovery, there is a lack of a consensus as to its efficacy which may primarily be due to differences in exercise modality, HWI protocol and timing of recovery. Therefore, we sought to investigate the effect of HWI on acute physiological responses utilising ecologically valid protocols. The recommended acute physiological response measures and time points of assessment were used from the first chapter, as well as recruiting participants from the same criteria and employing an identical exercise session. The results of this study indicate that HWI is a viable method of heat therapy that can maintain the rise in intramuscular temperature following resistance exercise. We also noted a suppressed response of hsCRP at 24 h following the exercise bout in the HWI group. Despite these results, there were no clear effects of HWI on measures of muscle function, muscle soreness or other blood markers of muscle cell disruption or inflammatory processes.

Collectively, these findings suggest that (i) post-exercise increases in intramuscular temperature can be maintained through the superficial application of heat, (ii) maintaining elevated intramuscular temperature may manipulate inflammatory processes, and (iii) clear effects for recovery of muscle function and muscle soreness are independent of HWI-associated changes in intramuscular temperature. These results represent the first investigation into the acute physiological responses of a 'real-world' HWI protocol following resistance exercise, alongside the use of a trained cohort, applied exercise session and utilising good nutritional practice.

The third and final investigation of this thesis (Chapter 6) was designed to build on the findings of Chapter 5. In athletic environments, individuals engage in resistance training programmes, with acute physiological responses seen as playing a key role in instigating beneficial adaptations (Schoenfeld, 2012). Given the recent evidence to suggest manipulating acute physiological responses may detrimentally influence training adaptation (Peake, Markworth, et al., 2015), it is pertinent to understand the effect of regular post-exercise HWI alongside a resistance training programme, of which this was the first study to do so. This study utilised the same exercise modality and HWI protocol as Chapter 5, as well as using the same participants, with the study rolled out over a 10-week training period. The key findings related to training adaptation were that chronic post-training HWI (i) augmented long-term gains in 1 RM, (ii) had no effect on the post-training increase in lower body lean mass and (iii) reduced muscle soreness ratings at 48 h post-training throughout the programme compared to PAS. Additionally, (iv) PAS saw reductions in baseline CK and IL-6 compared to HWI, while there were no differences between groups for other measures. The key findings related to acute

physiological responses were that chronic post-training HWI (i) attenuated the increase in muscle soreness as well as markers of inflammation and muscle cell disruption in the acute post-exercise period following training compared to PAS, and (ii) enabled an enhanced recovery of MVIC from pre- to post-training. Additionally, (iii) PAS saw accelerated recovery of ISO peak force and RFD_{0-100}, as well as reductions in muscle soreness ratings from pre- to post-training. The findings of this study are the first to suggest that HWI may be implemented alongside resistance training to enhance aspects of post-exercise recovery and promote strength adaptations. Considering the current study employed a trained cohort, an applied resistance training programme and good nutritional practice, the findings present evidence that HWI may be of interest to athletic populations looking to gain a competitive advantage in the 'real world'.

7.2 Discussion

Several important points relating to the interpretation of these findings have been discussed within each experimental chapter. The following section aims to bring together these themes and provide further context and scope for application of the findings.

7.2.1 Mechanisms

Hot water immersion is thought to exert a physiological impact primarily through an increase in cutaneous and subcutaneous tissue temperature which induces peripheral vasodilation and a subsequent increase in blood flow (Wilcock et al., 2006). The research behind these proposed physiological responses has typically occurred in the field of physiotherapy or with techniques of heat application including ultrasound and heat packs (Bonde-Petersen et al., 1992; Knight & Londeree, 1980; Wyper & McNiven, 1976). Several studies have highlighted HWI as a potent method to increase intramuscular temperature (Morton et al., 2007; Myrer et al., 1994), which causes an increase in cutaneous blood flow, due to peripheral vasodilation (Bonde-Petersen et al., 1992; Knight & Londeree, 1979). However, numerous factors will also impact the change in intramuscular temperature with HWI including: temperature gradient, the surface area of heat application, duration of exposure, environmental conditions and body composition (Myrer et al., 1994). In Chapter 5 of this thesis, it was demonstrated that an ecologically valid HWI protocol could maintain an elevated intramuscular temperature following resistance exercise for up to 1 h post-exercise. For the first time, this confirms that the hypothesised physiological responses of heat therapy are applicable to a HWI protocol, exercise session and participant cohort that would likely be seen in athletic environments.

Following an elevated intramuscular temperature and blood flow, it is expected that HWI will increase the permeability of cellular, lymphatic and capillary vessels which may drive increased rates of metabolism, nutrient delivery and clearance of waste products (Baker et al.,

2001; Coté et al., 1988). These physiological responses have been linked with an accelerated exercise recovery and would likely influence acute physiological responses, however supporting evidence is scarce. In Chapter 5, it was shown that HWI suppressed the response of hsCRP at 24 h post-exercise. However, there were no clear effects of HWI on measures of muscle function, muscle soreness or other blood markers of muscle cell disruption or inflammatory processes. Despite the limited influence of HWI on exercise recovery, the acute physiological responses that were manipulated (intramuscular temperature, inflammatory response) may be relevant within the context of adaptation to resistance exercise. Subsequently, Chapter 6 highlighted that HWI augmented long-term gains in strength in response to a 10-week resistance training programme. Following the training period, HWI elicited an accelerated recovery of muscle function and soreness in the acute post-exercise period and attenuated the increase of markers of inflammation and muscle cell disruption following training compared to PAS (see Figure 7.1). Collectively, these findings suggest that at the end of a 10-week training programme, HWI manipulates acute physiological responses to hasten post-exercise recovery. This may have positively impacted an individual's ability to train in subsequent sessions, leading to an accumulated training stimulus that induced small but worthwhile improvements in strength.

A possible link between the application of heat therapy and the benefits on recovery/adaptation is the role of heat shock proteins. Heat shock proteins respond to stress within the body, and among their divergent functions, serve to protect cells from damage (Noble, Milne, & Melling, 2008; Ohno et al., 2010). Previous reports have suggested that pre-conditioning with heat therapy 24 h prior to a bout of eccentric exercise, accelerates the recovery of muscle function and reduces subsequent muscle soreness (Nosaka, Muthalib, Lavender, & Laursen, 2007). These findings compare closely with the acute physiological responses shown in Chapter 6 and suggest that it is possible that regular HWI pre-conditioned the muscle to subsequent exercise bouts, by reducing contractile and structural protein degradation (see Figure 7.1). This may also explain the lack of an effect of HWI on muscle function, muscle soreness and CK following a single bout of resistance exercise in Chapter 5, whereas beneficial changes in these acute physiological responses were noted following the resistance training programme in Chapter 6. However, Nosaka et al. (2007) did not measure heat shock proteins and it is therefore difficult to directly attribute the accelerated recovery to this mechanism. Especially as others have reported no change in heat shock protein content following a HWI protocol which elevated intramuscular temperature to levels comparable to that following a bout of exercise (Morton et al., 2007). Nevertheless, the effect of HWI on heat shock proteins and

resistance exercise recovery is equivocal and requires further clarification (McGorm et al., 2018). The possible role of heat shock proteins as a mechanism therefore remains plausible.

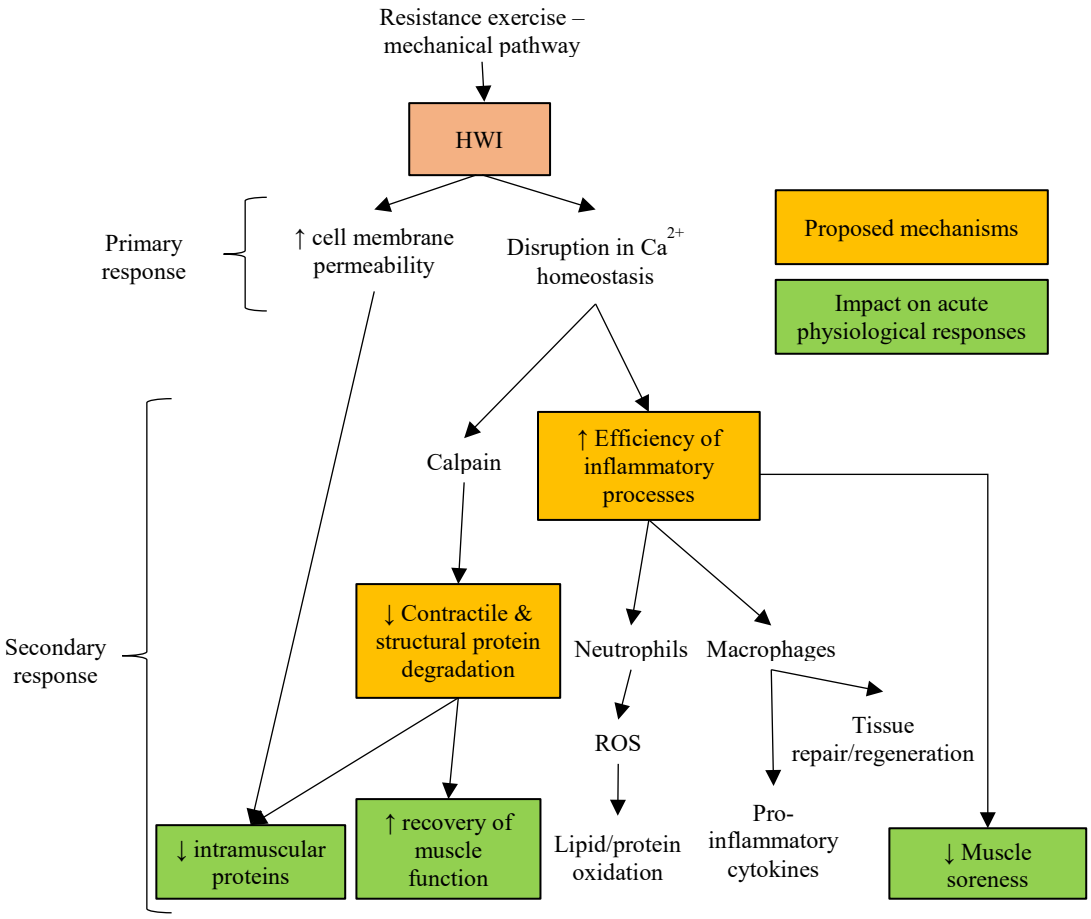

Figure 7.1 Proposed effect of regular hot water immersion (HWI) on acute physiological responses.

In addition to the role of HWI on acute physiological responses, there is also evidence of a direct role of heat therapy on other aspects that may beneficially influence training adaptation. For example, the increased intramuscular temperature and muscle blood volume associated with HWI (as reported in Chapter 5) would be hypothesised to aid muscle protein synthesis (Fujita et al., 2006). Increased tissue temperatures may promote rates of enzymatic processes, leading to enhanced glycogen resynthesis (Cheng et al., 2017), as well as upregulating key anabolic signalling pathways and protein expression following both passive treatments (Kobayashi et al., 2005; Uehara et al., 2004; Yoshihara et al., 2013) and with the presence of a mechanical stimulus (Goto et al., 2003; Kakigi et al., 2011). The heat shock response is also pertinent to this discussion whereby heat stress stimulates the expression of heat shock proteins which are known to play an important role during muscle protein synthesis (Goto,

2007). Therefore, several processes relevant to training adaptation may be stimulated by HWI and should be considered alongside the acute physiological responses presented in this thesis. This will help determine the relative contribution of augmented molecular responses vs. an enhanced recovery allowing a greater training stimulus in explaining the relationship between HWI and resistance training adaptation.

7.2.2 Recovery

As discussed in section 5.1, previous research related to the efficacy of HWI to enhance recovery following resistance exercise is equivocal. However, differences in exercise modality, HWI protocol and timing of recovery measures make conclusions problematic, lending to the assertion that further research utilising ecologically valid protocols are required in this area (McGorm et al., 2018; Vaile et al., 2010). In Chapter 5 of this thesis, we started to address this gap in the literature by showing that although HWI manipulated aspects of the inflammatory response, it did not impact measures of muscle function, muscle soreness or other blood markers of muscle cell disruption or inflammatory processes following a single bout of resistance exercise.

Resistance exercise is rarely performed as an isolated bout of exercise though and in Chapter 6, it was highlighted that HWI enhanced recovery following a session at the end of a 10-week resistance training programme. This included: an accelerated recovery of muscle function and soreness as well as attenuations in the rise of inflammatory and muscle cell disruption markers compared to passive recovery. These findings support those that have demonstrated HWI to enhance the recovery of jumping power during an intensified training week (Viitasalo et al., 1995). The authors attributed the beneficial effect to a positive influence on the neuromuscular apparatus and its functioning. An imbalance between the stress of training and recovery over extended periods may negatively impact performance (Barnett, 2006). As such, individuals may not be able to train at the required intensity or complete the next training session with an optimal load. The study design used in Chapter 6 most represents a real-life training environment that would be used by individuals engaged in regular resistance exercise (Halson, 2011). HWI may therefore be viewed as a training aid alongside resistance exercise to promote recovery, through favourable effects on acute physiological responses, during a training programme.

Further work should investigate the effect of HWI in other situations where the acute recovery between bouts of exercise may be optimised to result in accumulated benefits to performance e.g. competition/tournament scenarios and periods of intensified training. This will help to further elucidate the impact of HWI as a training aid for its application within applied practice.

7.2.3 Adaptation

It is perhaps unsurprising that given the paucity of literature providing a consensus for the effect of HWI on acute physiological responses, that research to investigate the long-term effects on exercise adaptation is scarce, to which there has been a call to arms from recent authors (McGorm et al., 2018; Méline et al., 2017). Therefore Chapter 6 of this thesis provides novel evidence to show that regular HWI can enhance strength development in response to a resistance training programme, although it had no effect on the post-training increase in lower body lean mass.

Chapter 6 highlights the potential benefit of HWI in accelerating the recovery following each session during a training programme, which is speculated to lead to augmented strength gains over time. While manipulations of acute physiological responses may be viewed as beneficial for post-exercise recovery, it is important to acknowledge that these processes are still required to stimulate adaptive processes (Schoenfeld, 2012). As reviewed in section 2.4.1, within the concept of hormesis, strategies may dampen or enhance the exercise response with the aim of optimising the adaptive stimulus. Post-exercise heat therapy has typically been viewed as a strategy to enhance the exercise stimulus within hormesis by applying an additional stress (Peake, Markworth, et al., 2015). However, this viewpoint has developed from various studies utilising animal models where the exercise modality and/or heat stress are not applicable to humans. The findings from Chapter 6 propose an alternative novel view of heat therapy, such that when applying post-exercise HWI as part of a resistance training programme, it is a strategy that may dampen acute physiological responses within hormesis, leading to a more optimal training stimulus with repeated sessions. Given that molecular signalling markers e.g. mTOR were not measured in this study, it is not possible to speculate as to whether these were up- or down-regulated. However, the influence of an accelerated recovery may outweigh these responses over a long-term training programme as individuals are able to repeatedly train with better quality. It should be noted that the response to different methods of heat therapy is likely context-specific and therefore application of the findings from this thesis should be applied as such. It is not ruled out that differing HWI protocols with regards to the temperature and duration may be associated with enhancing the exercise stimulus within hormesis.

Other strategies that may dampen physiological responses and reduce the exercise stimulus on the hormesis curve include: NSAIDs, antioxidant supplements and CWI (Peake, Markworth, et al., 2015). These strategies are purported to reduce symptoms of inflammation and/or oxidative stress to counter the associated deleterious consequences and accelerate recovery following exercise (Barnett, 2006; Bryer & Goldfarb, 2006; Leeder et al., 2011). However, recent reports suggest that the chronic use of these strategies may blunt long-term training adaptations (Michailidis et al., 2013; Roberts et al., 2015; Trappe et al., 2002). What

perhaps separates HWI from these strategies is that the underpinning physiological mechanisms are not speculated to reduce the inflammatory response *per se*. Rather it may be proposed that HWI assists a greater efficiency of response, via increased tissue blood flow, such that its resolution is accelerated. This provides the basis for the enhanced recovery between training sessions, for stimulation rather than attenuation of training adaptation.

Despite the potential benefits of HWI on strength development, it had no effect on the post-training increase in lower body lean mass. This is despite previous reports suggesting heat therapy to increase muscle cross-sectional area (Goto, 2007), as well as upregulate key anabolic signalling pathways and protein expression both independently (Kobayashi et al., 2005; Uehara et al., 2004; Yoshihara et al., 2013) and following a mechanical stimulus (Goto et al., 2003) in animal models. Although similarly to the present study, others have found neither a positive nor negative effect of heat therapy on resistance training-induced hypertrophy (Stadnyk et al., 2018). Perhaps the most compelling evidence in favour of heat therapy was found by Kakigi et al. (2011). This study demonstrated for the first time in human skeletal muscle that heat stress induced by a microwave therapy unit in combination with a bout of resistance exercise enhanced the phosphorylation of molecules within the mTOR pathway compared to resistance exercise in isolation. As discussed in Chapter 6, differences in participant training status, method of assessing hypertrophy, study design (animal vs human), method of heat therapy and timing of heat application may all contribute to discrepancies in the findings. Although HWI did not exert beneficial effects on hypertrophy within the context of this thesis, it may be possible that in other scenarios HWI may have potential hypertrophic effects, which is the subject for future investigation.

While the findings of Chapter 6 suggest a small, but worthwhile benefit of HWI for resistance-trained individuals during a training programme, further research is clearly required to investigate the efficacy of HWI in alternative scenarios. A point of consideration must be that although the findings of this thesis do not irrevocably support the use of HWI, it appears there is very little to suggest a detrimental impact of its use. While the reports of reduced muscle soreness in Chapter 6 indicate that it may exert perceptual benefits. For applied physiologists who must evaluate the pros and cons before implementing post-exercise strategies, HWI may offer potential benefits without any detrimental consequences.

7.2.4 Nutrition

Throughout each of the experimental chapters in this thesis, control procedures have been employed to account for any confounding effects in relation to nutritional intake and the associated impact on acute physiological responses. These include the use of food diaries, providing standardised meals and protein supplements. Although a thorough review of the

relationship between nutritional intake and acute physiological responses is outside the scope of this thesis, this section will address this area and provide rationale for the approaches used.

As discussed in Chapter 5, the addition of nutritional control approaches used by Roberts et al. (2015) may have contributed to discrepancies between the extent of the acute physiological response seen between Chapters 4 and 5. Previous research has demonstrated that consuming protein (in the form of branched chain amino acids) can attenuate reductions in muscle function, and reduce soreness as well as plasma levels of intramuscular enzymes in the post-exercise period in resistance trained individuals (Howatson et al., 2012). This may therefore explain the reduced response seen between Chapters 4 and 5. However, best practice recommendations (Jager et al., 2017) would suggest individuals to consume protein in the post-exercise period and therefore in line with the applied nature of this thesis, the inclusion of the nutritional control enhanced the ecological validity of Chapter 5 in this regard.

Protein supplementation has also been shown to augment increases in muscle size and strength following resistance training (Cermak et al., 2012). To maximise the post-exercise stimulus, it is important to ensure adequate protein intake, without which might elicit suboptimal adaptations. Therefore, to control for differences in protein intake between individuals, Chapter 6 included protein supplementation around each training session, as used by (Roberts et al., 2015). This approach, which would enhance the ecological validity of the study, would also reduce the window for an effect of HWI. Despite this, several positive findings were noted which highlights the potential application of this strategy in the real-world.

Other aspects of nutritional intake that were controlled throughout this thesis and have been shown to influence recovery from and adaptation to resistance exercise include: other nutritional supplements (Lanhers et al., 2015), alcohol (Parr et al., 2014), and NSAIDs (Mikkelsen et al., 2009). Although these may often be used by those partaking in regular resistance exercise programmes, it was important to control for these confounding factors that may manipulate aspects of the acute physiological response.

7.2.5 Site Change

A change in sites occurred between the data collection periods for Chapter 4 and Chapters 5/6 which impacted upon decisions made related to this thesis and therefore requires discussion. This change in location was due to unforeseen circumstances outside of the control of any individuals associated with this thesis. The change in location meant that some of the data collection methods (e.g. 20 m sprint performance), equipment (e.g. isokinetic dynamometer) and analysis tools (e.g. blood sample analysis techniques) were not available afterwards, meaning alternative approaches were considered. Although this change resulted in some

inconsistencies between Chapter 4 and Chapters 5/6, every attempt was made to minimise any impact to the interpretation of the findings.

7.2.6 Limitations of Findings

It is noteworthy that the findings of this thesis are relevant to the context in which the study was intended, which was for use in applied settings. The design of the studies therefore reflects this to maximise ecological validity, although as with all scientific research, may lead to limitations in the interpretation of the findings. The following section discusses the limitations that are pertinent to this series of investigation.

Throughout all the experimental chapters in this thesis, participants were recruited from an identical set of criteria which included being male, aged 18-35 years and resistance-trained. While a homogenous cohort was beneficial in comparing the effect of HWI to the PAS group, the application of the findings to other groups must be used with caution. It has previously been reported that acute physiological responses differ depending on sex (Rankin et al., 2015), age (Howatson & Van Someren, 2008) and training status (Donges et al., 2010; Vincent & Vincent, 1997a). Future research should incorporate a variety of cohorts to expand knowledge of the efficacy of HWI in different environments.

A further context-specific aspect of this thesis is the exercise modality, to which all the experimental chapters utilised a bout of resistance exercise that was designed based upon literature recommendations. This method, which includes exercises commonly used in strength and conditioning programmes (Haff & Triplett, 2015), improves the level of ecological validity compared with those that have opted to induce a mechanical exercise challenge through exercise modalities such as eccentric actions on an isokinetic dynamometer (Byrne & Eston, 2002b; Miles et al., 2008) or downhill running (Sorichter et al., 2001; Touchberry et al., 2012). As discussed in section 3.5, control procedures were implemented to standardise exercise technique, although an aspect of applied resistance exercise sessions is that participants will have innate differences. It would be of interest for future investigations to study a range of exercise modalities that are used in applied settings to increase the scope of application for HWI.

The HWI protocol utilised in Chapter 5 and 6 employed a moderate water temperature (40°C) and duration of immersion (10 min) such that the protocol could realistically be implemented by applied sports scientists or even recreational exercisers within leisure facilities, enhancing the ecological validity of the findings. As discussed in section 7.2.3, differing the water temperature and duration of immersion may stimulate alternative outcomes. Therefore, the findings of this thesis should be considered with reference to the specific HWI protocol and may not be applicable to other methods of heat therapy. Future work is required to investigate

the effect of water temperature and duration of immersion on acute physiological responses. This will aid the optimisation of HWI protocols such that recommendations can be provided as have been done with CWI (Leeder et al., 2011).

As discussed in Chapter 6, the possibility for placebo effects associated with HWI is a limitation of this work. The justification for implementing a non-intervention control group was based on recommendations that this is required to fully elucidate the impact of HWI (White & Caterini, 2017). Belief effects can be powerfully ergogenic, and in the modern world of success-related outcomes, are harnessed to improve an individual's ability to perform (Halson & Martin, 2013). Therefore, if belief effects form part of HWI-related outcomes, this may be utilised as a positive. However, previous reports have demonstrated that benefits of cold water immersion, including the recovery of strength, are not greater than the placebo effect (Broatch et al., 2014). Therefore, determining what impact belief effects have on the role of HWI with future work is important so that applied physiologists can employ HWI with confidence as an evidence-based strategy.

7.2.7 Other Applications

The focus of this thesis was to investigate the effect of HWI on acute physiological responses following resistance exercise and the impact on recovery/adaptation. However, there are several other avenues that have shown promise when linked with heat therapy and therefore it is worth considering how HWI could be applied in these fields.

As discussed in section 7.2.1, heat therapy has been shown to upregulate key anabolic signalling pathways and protein expression (Kobayashi et al., 2005; Uehara et al., 2004; Yoshihara et al., 2013), which may confer advantages in maintaining muscle protein synthesis and muscle mass in the elderly, injured athletes and in those with muscle-wasting disorders. The increased intramuscular temperature and muscle blood volume associated with HWI (as reported in Chapter 5) would also be hypothesised to aid muscle protein synthesis (Fujita et al., 2006). This could have important implications for reducing the age-related effects of Sarcopenia, helping older adults remain active, thus enhancing quality of life (McGorm et al., 2018). Given that the bulk of this research has occurred in animal models or with alternative method of heat therapy, it remains to be confirmed if HWI could confer such benefits.

Heat therapy has typically been used in the field of physiotherapy, however much of the literature is based on anecdotal information (Wilcock et al., 2006). In Chapter 5 and 6 of this thesis, novel data is presented to demonstrate that HWI may manipulate aspects of the inflammatory response, possibly via elevated intramuscular temperature and blood flow. Although the acute physiological responses that occur following a bout of exercise would be markedly different to those after a muscular injury, these reports provide a rationale for further

investigation. If HWI could positively impact upon the co-ordination of inflammatory processes following injury, there is potential for an accelerated recovery and return to fitness.

Concurrent with Chapter 6 and the positive impact of regular HWI, long-term passive heat acclimation has demonstrated benefits including: improved vascular function (Brunt, Howard, Francisco, Ely, & Minson, 2016) and muscle contractile properties (Racinais et al., 2016), as well as promoting mitochondrial adaptations (Tamura et al., 2014) and capillary growth (Kuhlenhoelter et al., 2016). Together these reports suggest that heat therapy could have clinical relevance for a variety of populations with a limited capacity to exercise. It should be acknowledged that often these adaptations are obtained with a relatively large external heat load and therefore an understanding of the minimal stimulus is required (Racinais et al., 2016). This would provide insights for the potential role of HWI in eliciting these beneficial adaptations.

There are clearly several avenues by which HWI has potential to provide a positive influence outside the scope of this thesis. Only through a greater understanding of the role of heat therapy and its mechanisms will we be able to capitalise on the opportunity for this strategy to be used across a variety of populations.

7.3 Directions for Future Investigations

This thesis has identified several areas for future research which have been discussed throughout. As a summary, these include, but are not limited to:

1 What is the optimal HWI protocol (e.g. temperature/duration/timing) to manipulate acute physiological responses to aid recovery and/or promote adaptation following resistance exercise?
2 What is the minimum increase in intramuscular temperature following HWI required to elicit the cascade of physiological events that leads to an increase in tissue blood flow?
3 What are the mechanisms that result in the HWI-associated manipulation of the inflammatory response/muscle soreness following resistance exercise?
4 Can HWI be utilised following other exercise modalities to accelerate recovery and promote adaptation as part of an intensified training programme?
5 Is there a belief effect associated with HWI?
6 What is the contribution of HWI-associated accelerations in recovery for promoting adaptation versus other potential stimulatory effects (e.g. molecular signalling)?
7 Can HWI be used as a strategy to promote beneficial adaptations in those with a limited capacity to exercise (e.g. elderly, injured athletes, diseased populations)?

7.4 Conclusion

The aims of this thesis have been addressed in three experimental chapters which have provided an important contribution to the literature. Despite hypothesised benefits, previous reports have provided equivocal findings in relation to HWI and recovery following resistance exercise, with its effect on adaptation not investigated and mechanisms undetermined. It has been shown that a practical HWI protocol can elevate intramuscular temperature and blood flow as well as manipulate acute physiological responses following resistance exercise in a trained cohort. During a resistance training programme, HWI was able to offer a positive impact on aspects of acute physiological responses, leading to greater long-term gains in strength. Although the findings from this body of work provide insights to enhance knowledge in the area, there is further work to be done to optimise the HWI protocol and widen the scope of application to other cohorts and with different exercise modalities, as well as deepen mechanistic knowledge. However, the positive aspects that can be drawn from the findings of this thesis outweigh the lack of harmful effects to provide physiologists with rationale for utilisation of HWI alongside resistance training in their applied practice.

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9 Appendices

9.1 Appendix 1: Example Written Informed Consent Document

CONSENT FORM

Title of Project:

Name of Researcher: Joshua Jackman

1. I confirm that I have read and understand the information sheet dated ..... for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

3. I agree that this form that bears my name and signature may be seen by a designated auditor.

4. I agree that my non-identifiable research data may be stored in National Archives and be used anonymously by others for future research. I am assured that the confidentiality of my data will be upheld through the removal of any personal identifiers.

5. I understand that the blood samples I provide will be stored at Allianz Park, before being transferred to Hendon Campus, Middlesex University until further analysis. I understand that subsequent analysis will take place at the labs on the Hendon Campus and samples will be analysed for markers of inflammation (IL-6, IL-10, C-reactive protein, MMP-9) and muscle cell disruption (creatine kinase). I understand that all samples will be destroyed following analysis or within a maximum period of 5 years.

6. I agree to take part in the above study.

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of parent/guardian (if appropriate)	Date	Signature

1 copy for participant; 1 copy for researcher;

9.2 Appendix 2: Example Health and Training Questionnaire

Middlesex University

M01, Mezzanine floor

Allianz Park,
Greenlands Lane,
London,
NW4 1RL

Health Screen Questionnaire

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1. Are you: Male or Female (please circle)

2. What is your date of birth?

Day: _____ Month: _____Year: _____ and Age: _____ years

3. When did you last visit your doctor (please circle)? In the:

Last week Last Month Last Six Months

Last Year Over a year ago

	YES	NO
4. Are you accustomed to regular moderate intensity exercise?		
5. Are you currently taking any medication?		
6. Has your doctor ever advised you not to take part in vigorous exercise?		
7. Has your doctor ever said "you have heart trouble"?		
8. Has your doctor ever said "you have high blood pressure"?		
9. Has your doctor ever said "you have low blood pressure"?		
10. Have you ever taken medication for blood pressure or your heart?		
11. Do you feel pain in your chest when you undertake physical activity?		
12. In the last month have you had pains in your chest when not doing any physical activity?		
13. Has your doctor (or anyone else) said "you have raised blood cholesterol"?		

14. Have you had a cold or feverish illness in the last month?		
15. Do you ever loose balance because of dizziness, or do you ever lose consciousness?		
16. Do you suffer from back pain that may be made worse by physical activity?		
17. Do you suffer from asthma?		
18. Do you have any joint or bone problems which may be made worse by physical activity?		
19. Has you doctor ever said “you have diabetes”?		
20. Have you ever had viral hepatitis?		
21. Do you have epilepsy?		
22. Do you suffer from any neurological disorders or injuries?		
23. Do you suffer from any muscular or ligament disorder or injury?		
24. Do you know of any reason, not mentioned above, why you should not exercise?		
25. Have you been performing resistance exercise for at least 2 years?		
26. Do you currently perform at least 3 sessions per week?		
27. Do you currently perform at least 1 session per week that includes leg-based exercises?		
28. Are you familiar with the technique of a barbell squat?		
29. Have you ever suffered any adverse reactions to hot water?		
30. Do you currently take any non-steroidal anti-inflammatory drugs (e.g. paracetamol/ibuprofen) or other analgesics for pain relief?		
31. Have you participated in a clinical study or received investigational drugs within the last 30 days?		
32. Do you have a recent history (within the last year) of alcohol or other substance abuse?		
33. Are you a current vegetarian?		
34. Do you currently take any form of nutritional supplement (e.g. creatine, HMB etc)? If so, please provide details to the researcher.		
Please fill in any other details relevant to the above here:		

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Name (CAPS): _____

Signed: _____

Witness: _____

Date: _____

9.3 Appendix 3: Example Food Diary

Food Diary

Participant ID:

Food Diary (to include all food and drink consumed during the specified period). Ideally this diary will be kept in real time (so keep it with you to enable accurate reporting of intake). Where possible include amounts per serving e.g. table spoon/weight/amount (e.g. two slices). Include types of food e.g. lean mince, skimmed milk, granary bread. Include brand of food e.g. Heinz, Hovis, Tesco Finest. Include activity whilst eating, i.e. eating out, at work, at home etc. Include any additional information that will enable you to best replicate this intake on a subsequent occasion.

Restrictions

- Please avoid nutritional supplements (e.g. protein/creatine)
- Please do not eat or drink for at least 3 hours prior to visit 3

Please note that information about your diet will be treated in confidence

If you have any problems or questions, please contact the study principal investigator:

Josh Jackman

07840179447

j.jackman@mdx.ac.uk

Day 1: _____

Time	Food and drink intake	Activity
9am	*Example...* *1 sachet of Quaker Oats porridge with 180 mL semi-skimmed milk and 1 200g Arla Protein Raspberry yoghurt*	*At home, watching TV*

Day 2: _____

Time	Food and drink intake	Activity
9am	*Example...* *1 sachet of Quaker Oats porridge with 180 mL semi-skimmed milk and 1 200g Arla Protein Raspberry yoghurt*	*At home, watching TV*

Day 3: _____

Time	Food and drink intake	Activity
9am	*Example...* *1 sachet of Quaker Oats porridge with 180 mL semi-skimmed milk and 1 200g Arla Protein Raspberry yoghurt*	*At home, watching TV*

9.4 Appendix 4: Standardised Warm-Up

Exercise	Distance/Repetitions
Forward/backward shuttle run	10 m x 3 reps
Side to side shuttle	10 m x 3 reps
Forward/backwards lunge	10 m x 1 rep
Inch worms into spiderman	5 reps
Single leg RDL with high knee pull	5 reps each side
Bodyweight squat	5 reps
Glute bridge into heel walkouts	5 reps
Single leg glute bridge	5 reps each side
Jump squats	5 reps

RDL, Romanian deadlift.

9.5 Appendix 5: Pilot Testing Results

Measure	Baseline	24 h	48 h
Creatine Kinase ($U \cdot L^{-1}$)	233 ± 60	541 ± 92	506 ± 123
Muscle Soreness (% of line)	1 ± 1	48 ± 7	45 ± 11

9.6 Appendix 6: Visual Analogue Scale (200 mm)

Visual Analogue Scale

Instructions:

From a standing position, squat down to approximately 90° and stand back up. Immediately upon standing up, use a pen to mark on the below line how much soreness you felt in your legs during the squat. The further to the left you mark, the less pain, the further to the right, the more painful.

No Pain/Soreness

Pain/Soreness as bad as it could be