IMPACT INDUCED MUSCLE DAMAGE: PERFORMANCE IMPLICATIONS AND ASSOCIATED TIMELINE

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Abstract

For contact-sport athletes, performance is often impaired following exposure to match-play or high-intensity training conditions. This has previously been ascribed to the negative influence of exercise induced muscle damage (EIMD) which occurs following unaccustomed or strenuous exercise and results in skeletal muscle ultrastructural damage and delayed onset muscle soreness (DOMS). However, athletes involved in contact-sports are exposed to repetitive collisions that have the potential to also result in impact induced muscle damage (IIMD). Previous research has noted that the EIMD theory may not be applicable to other forms of muscle damage including that occurring from blunt force trauma. This supports the contention that IIMD is distinct from EIMD, with an independent time course of potential changes in symptomology and associated functional capacity which remains to be elucidated. Previous studies investigating IIMD have yet to distinguish between EIMD and IIMD and as such findings may be confounded by the presence of EIMD. From the wider literature, it appears that EIMD and IIMD may be divergent with differing aetiologies, effects on functional capacity, adaptations, and interventions that may attenuate or mitigate the damage based on their mechanism of action. Therefore, the aim of the present research was to generate IIMD in the absence of EIMD and characterise the implications on performance, markers of muscle damage and inflammation, and perceptual measures in a cohort of contact-sport participants. To do so, a novel collision simulator was developed and a protocol implemented based on an amalgamation of published animal contusion research and contact-sport match-play data. A cohort of 18 healthy contact-sport athletes completed a single-group time series with measures assessed at baseline (PRE) and immediately following (POST) the IIMD protocol, with repeat testing 24, 48, and 72 h following the IIMD protocol. The collision protocol exposed the participants to 26 collisions over 80 min with an 80 kg load, which the frequency and intensity prescribed based on published match-based research. Biochemical indices of muscle damage (myoglobin [Mb]) and inflammation (high-sensitivity C-reactive protein [hs-CRP]), ultrasound assessment of oedema, 15 m sprint performance, squat jump peak power (SJ-PP), perceived mood, subjective function and soreness were assessed. Five, 10, and 15 m speed and SJ-PP were impaired for 48 h following the IIMD protocol ($P < 0.01$). IIMD significantly altered perceived function and mood for 24 and 48 h, respectively ($P < 0.01$), whilst subjective soreness was elevated from baseline for 72 h ($P < 0.01$) following the IIMD protocol. No change in ultrasound-derived oedema, [CRP] or [Mb] was observed ($P > 0.01$), primarily due to high interindividual variability. For the first time, the present research has shown that IIMD generated in the absence of
exercise results in impaired performance including the ability to produce power and speed. Additionally, IIMD produced changes in systemic markers of muscle inflammation but not muscle damage, with the effect being highly variable between participants. Further, IIMD negatively influenced perceived mood, function, and subjective soreness. These changes were most pronounced in the 48 h following the IIMD protocol. In conclusion, the results of the present study suggest that symptomology and functional capacity impairment commonly attributed to EIMD amongst contact-sport participants is at least in part due to IIMD. Furthermore, the experimental protocol implemented in the present study was successful in inducing IIMD in the absence of EIMD and therefore may be a valuable model to further investigate broader aspects of IIMD, including recovery and mitigation strategies. A number of potential future research streams based on this research and the model described are discussed herein.
Statement of Original Authorship

The work contained in this document has not been previously submitted to meet the requirements of an award at this or any other higher education institution. To the best of my knowledge, this document contains no material published by another person, except where due reference is made.

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Chapter 1. Introduction

1.1 BACKGROUND

Team sports are the highest participation sports internationally. Whilst the physical demands of training and competition vary within and between sports, each is characterised by repeated bouts of high intensity activity (1). Unique among these activities are contact-sports such as Rugby League (RL), Rugby Union (RU), American Football and Australian Rules football, which are further defined by physical contact interactions between athletes. Given the intense physical demands of these sports, athletes are regularly exposed to skeletal muscle damage occurring in response to stressors such as high-intensity exercise and collisions (2-4). This muscle damage can vary in severity along a continuum from mild exercise-induced muscle damage (EIMD) through varying degrees of muscular strain, to a muscular tear, and significant contusion, laceration, and crush injury (5). For athletes, muscle damage has the potential to adversely affect performance on a similar continuum from mild discomfort with little to no negative performance impact, through to substantial discomfort and associated performance decrements with prolonged athletic recovery.

Following unaccustomed exercise, a sensation of pain or stiffness is often experienced that peaks during the subsequent 24-72 h and is known as delayed onset muscle soreness (DOMS) (6). A number of investigations have revealed that repeated exercise involving typically eccentric contractions whereby a muscle actively lengthens under tension, are primarily responsible for DOMS (7-9). These actions are typically associated with increased mechanical stress resulting in microstructural damage, and a delayed inflammatory response (10). However, pain can increase disproportionally to that of tissue damage and can occur in the absence of tissue damage (11). Collectively, this process, known as EIMD has been the subject of a considerable body of research investigated in human and animal models (8, 10). Previous exposure to exercise (i.e. ‘training status’) appears to be a contributing factor; with research identifying that previously non-damaging concentric and isometric exercise can lead to substantial damage after a prolonged period of unloading (12). This damage typically occurs
in response to rapid acceleration and deceleration movements (13), plyometric exercise (14) and wrestling (15, 16). The time course and possible contributing factors to this damage have been an area of significant investigation given their potential impact on exercise performance.

An additional source of muscle tissue damage for contact-sport athletes are the collision events that occur throughout the performance interactions such as tackling, running into contact with the ball as well as through contact with the playing surface (3, 17). Preliminary research indicates these interactions produce impact-induced muscle damage (IIMD) (2), resulting in attenuated performance (18), muscle soreness (19), and increased intracellular proteins indicative of muscle damage following match-play (2, 3, 17, 20). The magnitude and time course of changes following IIMD are poorly understood, limiting insight into the significance of IIMD on functional capacity and athletic recovery.

To date, research examining recovery from muscle damage in contact-sports has been undertaken following match-play or match-simulation protocols. This typically involves evaluation of the time course of match-play on influencing neuromuscular function, indices of muscle damage and inflammation, subjective soreness, and mood change (21-24). Given these parameters are likely to be influenced by both EIMD and IIMD, potential for conflation of their individual effects is likely. Therefore for the exploration of IIMD, it would appear pertinent to examine any potential effects in the absence of EIMD in order to minimise conflation. Indeed, a previous review of musculoskeletal injuries noted the progression of injury and the secondary inflammatory response appear to be divergent between EIMD and direct muscle trauma (i.e. IIMD) (25). Furthermore, Merrick (25) noted that the EIMD theory may not be applicable to other forms of muscle damage including that which occurs following blunt force trauma. These findings support the contention that IIMD is distinct from EIMD, with an independent, as yet undefined, time course of potential changes in functional capacity and symptomology including perceived soreness, and indirect indices of muscle damage and inflammation, amongst others.
1.2 SIGNIFICANCE AND INNOVATION

Investigations in the area of IIMD have thus far been limited to a small number of studies which have examined high intensity exercise with induced IIMD or explored relationships between collisions and muscle damage indices following match-play. Thus, the present research is significant and innovative from a number of different perspectives.

To date, research has primarily investigated IIMD in the context of match-play and drawn conclusions based on relationships between systemic markers and/or changes in functional capacity following match-play. This approach is somewhat limited as it can be influenced by confounding factors associated with EIMD, and causation cannot be implied. The development of a safe, experimental model of inducing IIMD, with a standardised and systematic approach in the absence of exercise and thus EIMD, would allow for the identification and quantification of performance changes that occur following trauma typically associated with match-play. Additionally, this model will demonstrate the typical timeline of symptomology changes following IIMD for a given population. This model will then be available as a platform to explore other aspects of recovery from IIMD, including strategies that may mitigate or attenuate damage and associated performance impairment, and hasten recovery of functional capacity. This insight will allow for the more effective prescription and individualisation of recovery strategies for contact-sport athletes and sports science practitioners. From an animal perspective, research has been used to determine the muscle cellular and phenotypical response to impacts, this has yet to be studied in humans, including any associated performance implications. In the animal literature, studies often use a collision model which results in incomplete recovery (26), suggesting the forces involved are far greater and consequently the damage response is in excess of that which is experienced during match play or training. The present innovative research modifies this method to make it specific to contact-sport populations by prescribing impact loads based on reported game-related data from the wider literature (27). As such the present research is significant and innovative, as it is the first to independently assess the effects of impacts in an experimental, systemized manner based on actual game-related data.
1.3 GENERAL NAVIGATION OF THE THESIS

This thesis will introduce the reader to EIMD and IIMD, critically review and explore the literature relating to the aetiology of EIMD and IIMD, the effects of EIMD and IIMD on functional capacity and the timeline of impairment, the potential adaptation to a bout of muscle damage, and the identification of recovery strategies that may attenuate EIMD and IIMD. The thesis then details the methodologies of a study undertaken examining the effects of IIMD on select measures of performance, indirect indices of muscle damage and inflammation, and subjective pain, mood and function in the 72 h following a novel intervention to somewhat simulate collisions experienced during a rugby game. Furthermore, discussion surrounding the interpretation and relevance of these results as they relate to the current body of literature is explored. Finally, relevance of the current investigation against the body of existing literature is made, including recommendations for future research in this area.

1.4 AIM OF THE RESEARCH

The aim of this research is to evaluate and characterise the timeline of changes in response to an intervention designed to produce IIMD, in the absence of IIMD, in a cohort of contact-sport athletes. To appropriately characterise the response to IIMD, the changes in several parameters including performance based metrics, inflammatory and muscle damage indices, and mood/perceptual responses are to be examined. The findings may provide practitioners with further insight into the recovery process following IIMD.
Chapter 2. The Literature Review

The aim of the present review is to critically evaluate the literature on the aetiology of IIMD, effects of IIMD on functional capacity and the timeline of performance changes, and the potential adaptation to a bout of IIMD. To give context to IIMD, consideration is first given to EIMD, with emphasis on the degree of impact and timeline of changes across a range of measures including blood markers of muscle damage and inflammation, plus neuromuscular performance qualities, which have been explored in far more detail than the preliminary research investigating IIMD. Interventions proposed to favourably impact EIMD are also considered, given at least a proportion of these may have application in the management of IIMD. However, recognition is given to the fact that EIMD and IIMD may have divergent aetiology, and time course of impact on functional capacity. Where available, athlete data is examined in this context and where contact-sport athlete data is lacking, animal data is explored.

2.1 Exercise-Induced Muscle Damage (EIMD)

2.1.1 Aetiology of EIMD

Following unaccustomed or strenuous exercise, a sensation of pain or stiffness is often experienced that peaks in the 24-72 h after exercise. This is known as delayed onset muscle soreness (DOMS) and was first identified in a classic paper by Hough published in 1900 (6). Since then, a number of investigations have revealed that repeated unaccustomed exercise, typically eccentric contractions whereby a muscle actively lengthens under tension, are responsible for DOMS (7-9). These actions are associated with damage to skeletal muscle via a bi-phasic model by; increased mechanical stress resulting in microstructural damage, and a secondary, delayed inflammatory response (10). Collectively, this process is known as exercise-induced muscle damage (EIMD) and a considerable body of research has investigated human and animal models of EIMD (8, 10). The time course and possible contributing factors to this damage have been an area of significant investigation and are discussed here.
Amplified soreness, myofibrillar disruption, and release of intracellular proteins into the bloodstream have been identified following EIMD (8), resulting in decrements in muscle function and athletic performance (28). The possible causative factors associated with EIMD have been reviewed previously (8, 29, 30). However, the magnitude and progression of EIMD is highly variable between and within individuals with observations ranging from a negligible amount of necrotic fibres, to extensive tissue necrosis and inflammation (8). Factors which influence the intra-, and inter-individual response include sex (31, 32), genetic factors (33), age (34), prior exposure to the muscle damaging activity (35) and hydration status (36).

Novel or unaccustomed exercise is thought to result in EIMD via a bi-phasic mechanism; i) a primary mechanism originating in damage of the ultrastructure of the muscle tissue, including the contractile apparatus with compromised membrane integrity, and the subsequent release of intracellular muscle cell-specific proteins into the blood stream (25), and ii) a secondary mechanism characterised by the inflammatory response that results from the binding of neutrophils, macrophages, cytokines and other pro-inflammatory markers to the area of damage (37). Pain develops and is amplified via sensitizing of type III and IV afferent nerves to prostaglandins released from the damaged cells and from the movement of fluid and cells into the interstitial spaces resulting in oedema (9). The cascade of events that follow EIMD are primarily driven to remove, regenerate, and finally remodel damaged tissue and appropriately adapt for future exposure to potential muscle damaging exercise. Thus, these processes can be seen as physiological appropriate measures to stimulate the healing response.

Primary damage (disruption of cellular constituent proteins such as Z-lines, troponin, tropomyosin and actomyosin bonds) is thought to occur following eccentric contractions as these actions produce a higher tension per unit of cross-sectional area than other contraction types (16, 37). On repeated contractions, this increased mechanical stress results in increased membrane permeability and physical damage to the macro- and micro-structural elements of the muscle (6). This ultrastructural damage and increased muscle fibre permeability in turn leads to the loss of muscle-specific proteins and increased presence of these proteins within
the bloodstream (38). Previous research has investigated markers such as concentrations of creatine kinase ([CK]), and myoglobin ([Mb]) as indicators of muscle damage following EIMD (39, 40). Similarly, concentrations of biomarkers of inflammation including high-sensitivity C-reactive protein ([hs-CRP]), Interleukin-6, and tumour-necrosis factor α have been previously used as monitoring tools following EIMD (7, 10). Whilst these biomarkers have been used extensively to characterise EIMD, typically their systemic concentrations poorly mirror the damage progression over time, and are influenced by the timeline of release from the muscle and subsequent removal from circulation (40). However, relationships between a selection of these markers and collisions have been observed following match-play (17), and as such they may offer improved utility in examining other forms of muscle damage, such as IIMD.

The secondary phase of muscle damage is a complex process which involves the concert of inflammatory and immune systems. Initial damage leads to the deformation of T-tubules and damage from the opening of stretch-activated ion channels and the resultant calcium flux (37). Thereafter neutrophils migrate to the area of damage and in turn, lead a pro-inflammatory cascade to that area through the secretion of proteases, enzymes that further break down the cellular structural proteins into their constituents (41). Proteases assist in the removal of damaged cellular components (protein metabolites released following ultrastructural damage), sometimes resulting in damage to healthy nearby tissue (37, 41). Local neutrophil effects are mediated through the production of reactive oxidant species (ROS) which play a key signalling role in the inflammatory response to exercise (42). Following the initial 24 h, neutrophils are substituted for macrophages that remove cellular debris via cytotoxic induction of the nitric oxide (NO) pathway (41). Towards the final stages of secondary damage, these macrophages shift to a phenotype that activates satellite cells in the vicinity to the presence of damaged tissue, which begin the regeneration and remodelling of muscle fibres (43). Throughout the secondary inflammatory phase, it is apparent that interactions and integration between the inflammatory and immune systems is necessary for regeneration and recovery of the skeletal muscle structure.

Whilst ultrastructural skeletal muscle damage has historically been viewed as the source of DOMS, recent work has suggested several other mechanisms such as damage to fascia and
neurotrophic factors may play a key role in the amplified soreness experienced following EIMD. Lau et al. (44) examined pain responses following exposure to an eccentric exercise, muscle damaging protocol of the elbow flexors. They measured pain using an electrical pulse algometer placed within the biceps brachii and brachialis muscle bellies and at their individual fascia prior to, and in recovery from a muscle damaging exercise protocol. Pain responses in to stimulation in the fascia were significantly greater than those within the muscle belly. This suggests that damage to fascia and connective tissue surrounding muscles may be partly responsible for activation of the nociceptors and the induction of pain and discomfort that is known as DOMS. A recent review of animal models of EIMD has identified neurotrophic factor pathway’s which may lead to increased pain and soreness in the absence of ultrastructural damage (45). Mizumura and Taguchi (45) examined several recent animal studies and found the presence of hyperalgesia (soreness following EIMD) linked to a bradykinin-nerve growth factor dependent pathway and a cyclooxygenase (COX)-2 and glial cell line-derived neurotrophic factor (GDNF) pathway. A recent investigation by Hayashi et al. (11) has confirmed this by finding significant large correlations between the concentrations of these neurotrophic factors and the degree of hyperalgesia, in the absence of muscle fibre ultrastructural damage. Taken together, these findings suggest a direct mechanism for neurotrophic factor control of peripheral pain and soreness following EIMD which can occur in the absence of intramuscular damage.

In summary, the mechanisms responsible for EIMD and the associated symptomology appear to be mediated through the progression of primary ultrastructural damage and secondary inflammatory processes. Primary structural damage occurs to the muscle fibre and membrane constituents through mechanical stress, and secondary damage occurs via changes in the cellular milieu derived from inflammatory and immune system processes. Recent research suggests that fascia and neurotrophic factors may play an important role in the amplified soreness in the absence of ultrastructural damage. The current research suggests that the individual response to EIMD exists on a continuum (8), where the adaptive or maladaptive responses to the primary and secondary mechanisms are modulated by factors such as the mode of muscle activation, training status of participant, novelty of exercise, and individual genetic variability (7). Therefore, it is important for athletes and practitioners to understand this continuum of damage and effects on the individual.
Understanding these physiological mechanisms responsible for muscle damage affords an opportunity to propose and explore interventions which may mitigate, treat or manage that damage based on the proposed mechanism of action of each intervention.

2.1.2 EFFECTS OF EIMD ON PERFORMANCE

From a neuromuscular function and athletic performance perspective, reductions in force production capacity following EIMD is a reliable indicator of the severity of muscle fibre damage (46). Similar to the magnitude of observed physiological damage, the magnitude of force losses following EIMD are dependent on factors such as the mode of muscle activation, genetic differences, the muscle or group of muscles impacted, and the novelty of the exercise (8). Although the magnitude and timeline of performance changes have been explored, the mechanism(s) by which EIMD causes decrements in the ability to produce force is poorly understood, with the majority of evidence being derived from animal models. The effect of EIMD on neuromuscular function has been broadly attributed to two mechanisms; the progressive disruption of sarcomeres, and disturbed excitation-contraction coupling triggered by altered calcium flux.

The decline in ability to produce muscular force in the days following eccentric and/or high intensity exercise is a consistent and robust marker of tissue damage (8). Whereas other measures such as soreness, [CK], and swelling do not correlate particularly well over time with histological evidence of damage, force loss is generally representative of EIMD severity (46). Impaired force production with the range of 15-60 % has been observed for up to 7-14 days following EIMD (8, 46). The magnitude of changes following EIMD is mediated by the interplay between mode and intensity of exercise, in addition to the novelty of the stimulus. For example, research has identified that force loss following eccentric actions of the biceps brachialis (50-60 %), are much higher than the 10-30 % loss reported following eccentric action of the quadriceps femoris (such as downhill running) (8, 47). This is potentially due to an adaptation to regular exposure of the lower limbs to eccentric exercise through everyday ambulation (discussed further in Chapter 2.1.3). Furthermore, induction of EIMD through isolated eccentric exercise or isokinetic dynamometry produces greater force decrements.
than methods such as downhill running (8). In some individuals, strength losses have exceeded 70 % of baseline values and not returned to baseline for upwards of two weeks following injury. Genetic variability in the primary and secondary damage response has recently been implicated as responsible for this discrepancy (7).

The timeline of performance changes following EIMD typically displays a robust and consistent pattern. Strength and power losses are greatest immediately after EIMD and in the first few days before returning to baseline over the subsequent seven to 14 days (39, 40, 46). For example, Evans et al. (39) observed significant decrements in eccentric torque which peaked at 0 h (43.5 %), and remained compromised at 24 (38.8 %) and 48 h (32.0 %) following eccentric contractions using isokinetic dynamometry of the elbow flexors. Fourteen days after the completion of the exercise task, participants’ eccentric torque had returned to baseline. These findings are in agreement with other research that has observed a loss of concentric and eccentric torque peaking immediately following exercise before a return to baseline by four to seven days following exercise (47). Similarly, reductions in maximal voluntary contraction (MVC) torque capacity and isometric strength are typically greatest immediately following EIMD before returning to baseline four to six days following exercise (40). Finally, EIMD results in significant reductions in range of motion (ROM) in the damaged limb, resulting from oedema in the myotendinous junction and in the connective tissue surrounding the muscle related to the secondary inflammatory response (39). Peak impairment in ROM is typically observed 24-48 h following EIMD before returning to baseline over subsequent days (40). Taken together, these data highlight that in most performance measures, peak decrements are observed in the first 24-48 h following EIMD before returning to baseline over subsequent days.

The theory of sarcomere disruption has been proposed as a source of functional strength deficits observed following EIMD (48). This theory suggests that during active lengthening, the weakest half-sarcomeres take up much of the length change, progressively stretching beyond actin and myosin filament overlap, resulting in ‘popping’ or disruption to these weak sarcomeres (48). While the majority of sarcomeres resume normal function at the cessation
of active tension, an increasing amount become disrupted, with each repeated eccentric contraction. Similarly, disturbed excitation-contraction coupling has been suggested to negatively influence force generation capacity following EIMD. The structural damage to the sarcolemma following EIMD leads to partial inactivation of the associated sarcomeres and in turn, a reduction in tension (48). The damage to the ultra-structure produces changes in ionic flow through stretch-activated and leak channels (49), which leads to an influx of calcium and sodium ions into the muscle fibres. As a consequence this alters the membrane potential, resulting in a reduction in the amplitude of the action potential and therefore, of calcium release in the proximity of the contractile apparatus (49). Finally, calcium-mediated proteases accumulate within the muscle in response to the increased intracellular calcium flux and increase lysis of structural proteins, further compromising force generation capacity (8). These processes lead to reductions in the ability to produce force at a cellular level as a result of damage to the T-tubule and resultant disturbed ionic flux.

2.1.3 ADAPTATION TO EIMD

An initial bout of muscle damaging exercise has been identified to confer a protection against the effects of a similar intensity, subsequent bout of exercise. This phenomena is known as the repeated bout effect (50) and has been studied extensively. The repeated bout effect was first identified by Highman and Altland (51) and has been identified following high intensity exercise and eccentric muscle actions in both animal and human models (50). The benefits of this adaptation can persist for up to six months following an initial bout of muscle damaging exercise, with significantly reduced muscle damage indices such as reductions in force loss, muscle damage markers, and soreness following the secondary bout (46). Whilst the exact mechanism(s) for this adaptation remain unknown, it has been broadly attributed to a number of factors including neural, mechanical, and/or cellular adaptations (35).

Presently the exact mechanism for the adaptation associated with the repeated bout effect is poorly understood. In their most recent review on the mechanism of adaptations, McHugh (35) discussed the proposed adaptation that occurs from a neural, mechanical, and cellular
adaptation perspective. They note that there is varying evidence which may at times seem contradictory to these proposed models. This may be partially driven by varying protocols used to induce EIMD resulting in different adaptations. For example, research has shown a repeated bout effect following passive stretching and exposure to two eccentric contractions which resulted in minimal signs of damage (52). The implemented exercise mode used was unlikely to result in the longitudinal addition of sarcomeres, one proposed cellular mechanism. However, the initial exercise exposure may have initiated an adaptation in the inflammatory response and served to limit the subsequent secondary damage (35). Another theory suggests that damage to stress-susceptible fibres following an initial bout may facilitate their removal and replacement with stronger fibres, whilst remodelling of extracellular matrix proteins following EIMD has been highlighted as a contributing factor (8). Currently a unifying theory remains elusive and it is likely that there are several mechanisms that appear to either complement each other or operate independently.

2.1.4 EIMD AND RECOVERY STRATEGIES

Strategies to assist recovery following exercise have been an active area of research with a number of interventions explored for their efficacy in preventing or facilitating recovery following EIMD. Proposed strategies include cryotherapy (53), hydrotherapy (54), compression garments (55), therapeutic ultrasound (56), and dietary interventions such as antioxidant (57, 58), and protein supplementation (59, 60). A number of these strategies have been identified to improve recovery from EIMD including cold-water immersion (CWI), compression garment use, and supplementation with cherry juice, although results are at times inconsistent (5, 61). These strategies have focused on targeting aspects of primary damage or the secondary inflammatory response as a means to mitigate damage or facilitate recovery from EIMD.

Cryotherapy

Cryotherapy, defined as body cooling for therapeutic purposes, has been applied in sports and exercise medicine using various mediums (53). Cryotherapy has been recommended for
its supposed effects including decreased cellular metabolism, reduced tissue necrosis and apoptosis (62). Primarily, cryotherapy achieves its clinical effect by peripheral vasoconstriction and decreasing muscle temperature, with animal models reporting a temperature decrease of 5-15 °C as optimal for reducing cellular metabolism, and associated secondary damage (25). However, in healthy human models, cryotherapy in the form of topical ice or CWI has more often than not failed to reduce temperatures to these levels, with ~21 °C being the lowest reported intramuscular temperature following crushed ice application (62). This suggests that the majority of these studies have been unable to decrease temperature to the threshold necessary to see benefit, with ergogenic influences possibly reflecting a perceptual or placebo effect (63). Additionally, strategies that have demonstrated only small effects may be more effective in combination with other strategies as a means to compound their effects (e.g. topical ice application and compression).

A recent development in cryotherapy has been the use of whole-body cryotherapy (WBC) involving exposure to extremely cold dry air (typically -100 to -140 °C) (53). Given the novelty of this intervention, studies exploring WBC are limited and have typically involved small sample sizes (64-67). A study by Hausswirth et al. (66) identified attenuated [CK] following daily WBC in recovery from EIMD. A recent review noted that repeated WBC may improve subjective soreness and recovery, similar to other methods of cryotherapy, with little evidence that these benefits translate to functional recovery (53). However, these studies were undertaken using a cross-over design methodology and featured a short period of weeks between treatment and control conditions. Thus, the results were likely influenced by the repeated bout effect. Russell et al. (68) investigated the effects of a single bout of WBC compared to passive recovery following muscle damaging repeated sprints. They identified no significant differences in [CK], peak power output, or perception of soreness and recovery compared to control. Further research is needed to ascertain if WBC is an effective treatment strategy following EIMD, and if so, the timing and dose with which it remains effective.

An important consideration for practitioners when applying cryotherapy is considering the context and aim within which these strategies are implemented. For example, the use of CWI
following acute resistance training has been associated with improvement in ability to complete additional work in subsequent training sessions, possibly resulting in improved long-term adaptations (69). However, the chronic use of CWI following resistance training has been shown to significantly decrease long-term gains in strength and hypertrophy (70). This was mediated through a decrease in activation of signalling proteins and satellite cell activation following CWI. Conversely, acute and chronic post-exercise cooling has been found to promote mitochondrial biogenesis markers such as peroxisome gamma coactivator 1-α (PGC-1α) (71, 72) following endurance exercise training. These findings suggest CWI may be an effective strategy to promote adaptations to endurance training, although the effect of CWI on endurance performance has not been examined. From this perspective, the context in which CWI is applied needs to be addressed by practitioners with a possible adaptive role in endurance training adaptations, and a maladaptive role in resistance training adaptations. Overall, CWI should be strategically scheduled to manage damage and inflammation in an acute context when recovery between sessions is limited, while not adversely impacting the adaptive response.

**Hydrotherapy**

Various methods of hydrotherapy, such as contrast water therapy (CWT) have been explored for their effectiveness in promoting recovery following EIMD. CWT involves alternating between cold- and warm-water and has been extensively used within the sporting community (73). It has been suggested that CWT reduces oedema through a “muscle-pump action” of alternating peripheral vasoconstriction during CWI, and vasodilation during warm-water immersion (WWI) (74). CWT has been postulated to assist in the removal of muscle metabolites and reduce secondary inflammation (74), possibly promoting recovery. An increase in hydrostatic pressure with associated osmotic shift from peripheral to central compartment has also been proposed. Vaile et al. (73) observed decreased localised swelling at 0 and 48 h following CWT after eccentric exercise. Squat jump peak power and peak isometric squat force were significantly improved following CWT when compared to passive recovery. These findings were further validated by research which found CWT to improve recovery of isometric peak force and peak power following a bout of eccentric exercise, compared to passive recovery (75). Similar results were observed following CWI, however,
following WWI recovery of isometric peak force was improved but jump squat peak power was not. Collectively, these findings support both CWT and CWI as interventions for attenuating muscle damage indices following eccentric exercise. The greatest benefits for athletic recovery have been observed following immersion in 10-15 °C water for 10-15 min (76), although individual prescription may be necessary (77).

A systematic review and meta-analysis by Bieuzen et al. (74) examined CWT compared to passive recovery, CWI, and WWI in the treatment of EIMD. Compared to passive recovery, CWT produced improved recovery of strength at all follow-up time points to >96 h following EIMD. Similarly, compared to passive recovery, CWT resulted in significantly lower levels of DOMS for >96 h following EIMD. However, indices of muscle damage were significantly reduced at 48 and 72 h post-exercise, with no difference in indices of inflammation at any time point following CWT (74). In comparing CWT to other treatment interventions, it was noted that the evidence showed only small and inconsistent effects, suggesting that there is little evidence for a superior treatment effect between CWT and other popular recovery interventions (74). These findings also highlight the potential confounding role that the placebo effect has on the application of recovery strategies, and the difficulty of blinding participants to the treatment in recovery research (55, 74, 78).

Compression

Compression garments have been widely used to treat clinical pathologies such as deep vein thrombosis (79, 80), with their use in sporting contexts as a means to promote recovery increasing in recent years. This increase in application has been driven by the purported benefits of compression creating pressure gradients which enhance blood flow, facilitating the removal of muscle metabolites and waste products (55). In this manner, compression garment use targets the secondary inflammatory response by reducing oedema, thereby reducing osmotic pressure on nociceptors and attenuating the experience of pain. A recent investigation by Valle et al. (81) examined the effect of compression worn during 40 min of downhill running on a motorised treadmill. Comparison was made using a within-subject
design with a specialised garment covering one thigh with the contralateral uncovered thigh acting as control. Histological evaluation of biopsy samples taken 48 h following EIMD identified significantly greater intracellular infiltrate of extracellular proteins and inflammatory cells in the control limb compared to compression limb. These results indicate compression garments can decrease damage and improve recovery at the cellular level by decreasing the cellular infiltrate associated with primary and secondary damage (81). While the benefits of improved cellular recovery on subsequent functional capacity were not examined, the effects of compression on performance have been investigated in recent reviews.

Compression garment use to promote recovery from EIMD has been the focus of two recent systematic reviews and meta-analysis’. Hill et al. (55) examined the effects of compression on the recovery of DOMS, strength and power, and [CK]. Compression garments were identified as having a moderate effect in improving DOMS (Hedges’ $g = 0.403$), muscular strength ($g = 0.487$), and power ($g = 0.487$). Similarly, compression garment use was found to have a moderate effect on systemic [CK] ($g = 0.439$). The results should be interpreted with caution as one study (82) observed a much larger effect ($g = 3.835$) on [CK] than the other studies which observed much smaller effects ($g = 0.025 – 0.465$) (83-86). The inclusion of this study likely skewed the data and findings (55). Overall these results show that compression garment use may be an effective strategy in the attenuation of functional capacity indices following EIMD. These findings were supported by Maques-Jimenez et al. (87) who identified strength and power measures to recover faster following compression garment use. In their review, compression was identified to elicit a small improvement on [CK]. Collectively, these results highlight the variability in the CK response to EIMD and question the reliability of using CK as a monitoring tool following EIMD (40). From these reviews, compression garments appear to hold promise as a strategy to positively influence performance recovery and DOMS following EIMD, with a small yet inconsistent effect on [CK].

Therapeutic ultrasound
Therapeutic ultrasound is thought to promote recovery by increasing blood flow and consequently reducing oedema and secondary damage (56), and has also been investigated in animal contusion research models (88, 89). Given its purported mechanism, it utility may be of relevance to facilitating recovery following EIMD, although research in this area is limited (56). To the author’s knowledge, there is only one study so far which has examined ultrasound as a treatment strategy following EIMD (56). Application of ultrasound for 20 min was found to decrease soreness following EIMD. Clearly more research is needed to investigate the effects and mechanisms of ultrasound therapy in EIMD.

Dietary strategies

Dietary strategies have shown promise for attenuating indices of muscle damage following EIMD. Polyphenol rich food sources have been identified as an effective strategy to hasten recovery via antioxidant and potentially anti-inflammatory pathways (90-92), whilst supplementation with antioxidants such as Vitamin C and Vitamin E have been investigated for their effects in improving recovery (93-95). Similarly, protein ingestion has been investigated as it may provide benefit in aiding recovery from EIMD with a potential role in the preservation of intracellular structural proteins (96, 97). In this manner, supplementation is purported to attenuate primary damage to the ultrastructure, with limited evidence of an influence on the secondary inflammatory response. Previous research has examined the benefits of dietary protein consumption and the dose and timing at which they are most effective following EIMD. Finally, consumption of omega-3 polyunsaturated fatty acids and curcumin have been investigated for potential roles in promoting recovery (98-102).

The progression of damage following EIMD involves the production of reactive oxygen species (ROS), which impose an oxidative stress upon the tissue (10, 103). Accumulation of ROS can further damage the ultrastructural constituents and has been implicated in the secondary damage that follows primary membrane disturbance (56). Antioxidant compounds are known to reduce ROS and therefore decrease the oxidative stress imposed upon the tissue (56, 104). Against this background, a number of studies initially examined supplementation with antioxidants Vitamins C and E, either separately or in combination, in preventing or
attenuating EIMD. A study by Bryer et al. (105), investigated the effects of 3000 mg·day$^{-1}$ Vitamin C supplementation for 14 days prior to EIMD found supplementation decreased muscle soreness in the first 24 h following exercise. These findings were supported by a study investigating 3000 mg·day$^{-1}$ of Vitamin C for three days prior to and four days following EIMD, and found that supplementation decreased soreness (56). In contrast, an investigation utilising a near-identical research design found no effect of Vitamin C on muscle soreness, strength loss or ROM (106). The literature investigating Vitamin E supplementation is similarly unclear (56). When exploring antioxidant supplementation, one important consideration is the potential for supplementation to decrease endurance training adaptations (107). Gomez-Cabrera et al. (108) explored supplementing 1 g of Vitamin C taken daily during an eight week endurance training programme against control. Compared to the control group, supplementation with Vitamin C decreased markers of mitochondrial biogenesis such as PGC-1α and decreased gains in endurance capacity. From an acute ingestion perspective, similar adverse effects have been observed with Vitamin C supplementation prior to exercise training, negatively influencing running speed (109). Collectively, these studies highlight the equivocal nature of the wider literature, with a review further underlining an inconsistent effect of Vitamin C and E supplementation on recovery following EIMD (56). Differences in the dose, supplementation period, and exercise interventions are possible underlying factors which may explain at least part of the variance in these findings.

Given the uncertain nature of the effect following supplementation and the potential for supplementing with antioxidants to reduce training adaptations, a shift towards examining food sources rich in antioxidant compounds known as polyphenols has been undertaken to explore their potential role in attenuating EIMD (56, 90, 110, 111). Polyphenols are a class of organic chemical compounds found mainly in plants, and have been found to exhibit antioxidant and anti-inflammatory properties in human and animal models (110), and include quercetin, resveratrol, and catechins, amongst others (57). A number of studies have investigated consumption of foods rich in phenolic compounds known as anthocyanins, to prevent EIMD. Initial work by Connolly et al. (111) explored the effects of tart cherry juice supplementation or placebo for four days prior to, and four days following EIMD. Supplementation resulted in significantly lower strength loss and soreness compared to
placebo. This work was supported by a study by Howatson et al. (90) who investigated cherry juice supplementation for five days prior to, and in two days following completion of a marathon. Strength recovery, inflammatory markers such as IL-6 and [hs-CRP], and circulating markers of oxidative stress were improved in the supplementation group compared to placebo.

Similarly, other polyphenol rich foods sources have been explored as dietary strategies for their potential effect in preventing EIMD (91, 112), although studies are limited. Blueberries are one potential source of polyphenol compounds such as anthocyanin and have been found to suppress oxidative stress induced muscle damage *in vitro* (91). A study by McLeay et al. (112) explored the effects of an anthocyanin containing blueberry supplement against placebo, on indices of muscle damage and performance following EIMD. Supplements were provided on the day prior to exercise and twice in the 36 h following exercise. Recovery of isometric torque capacity and plasma oxidative capacity were significantly greater in the supplementation group compared to control, whilst soreness, and markers of muscle damage and inflammation were not different between groups. Given that the research on blueberry supplementation is limited to these two studies, further research is needed to explore the potential benefit of supplementation, and the dose and timing of ingestion for that benefit to be realised. The proposed mechanism for these findings suggests cherries and blueberries and their associated phenolic compounds were able to mediate the secondary, inflammatory damage response through reducing ROS. This ultimately led to a reduction in some indices of muscle damage following EIMD. Tart cherry juice supplementation is one feasible and well established strategy to promote recovery from EIMD, with blueberry supplementation requiring further exploration.

Protein supplementation (59, 96, 97, 113, 114) has been extensively investigated in its potential to prevent or treat EIMD by targeting different aspects of primary or secondary damage. Ultrastructural damage is known to occur following EIMD, with enhanced rates of protein degradation likely to play a causal role in this damage to the force-generating and/or force-transmitting structures (59, 97). Therefore, consumption of optimal protein, known to
induce positive changes in skeletal muscular protein synthesis (115), has been suggested to minimise damage symptomology following EIMD. Cockburn et al. (97) investigated the effects of milk, a source of dietary protein and carbohydrate, and an isonitrogenous protein supplement against non-isocaloric placebo. They identified that milk and the protein supplement significantly attenuated peak torque, and markers of muscle damage 48 h following EIMD, with no difference in muscle soreness between groups. Further research by Cockburn et al. (96) examined the consumption of milk compared to non-isocaloric placebo (water) following EIMD, with findings suggesting milk consumption improved recovery of agility and 15 m sprint performance. There were no differences in countermovement jump performance, [CK] and [Mb], and subjective soreness between the groups. The purported mechanism for performance improvements was the increase in muscular protein synthesis due to supplementation resulting in improved maintenance of contractile proteins and cell membrane integrity, and a decrease in myofibrillar disruption (59). Increased synthesis of myofibrillar and membrane proteins improved the recovery of performance indices and in some cases decreased systemic blood marker concentrations following EIMD.

Additional research has explored the effects of timing and dose of supplementation on attenuating EIMD (113, 114). Protein supplement ingestion immediately following eccentric exercise was found to be beneficial in attenuating performance decrement, when compared to pre-exercise ingestion, and at 24 h following exercise. Finally, Cockburn et al. (113) investigated the ingestion of 500 mL semi-skimmed milk (17 g protein), 1000 mL of semi-skimmed milk (34 g protein), or non-caloric placebo (0 g protein) consumed immediately following EIMD. Consumption of 500 mL of milk reduced decrements in peak torque and [CK] compared to placebo, whilst 1000 mL consumption reduced IL-6 concentration compared to placebo, with no difference in passive or active soreness observed following either supplementation strategy. Overall, there was no difference between the either dose of milk when compared to placebo. This suggests that 500 mL of semi-skimmed milk was enough to attenuate aspects of EIMD. The 17 g of protein provided by the 500 mL milk drink is close to the 0.25 g·kg\(^{-1}\) body mass previously highlighted as being the breakpoint beyond which dietary amino acids are no longer incorporated into skeletal muscle in younger participants.
From a recovery from EIMD perspective, these studies suggest that consuming protein beyond this threshold does not provide any additional benefit in improving recovery.

Omega-3 polyunsaturated fatty acids have been investigated as a supplementation strategy to promote recovery from EIMD. Interest in omega-3 supplementation as a recovery aid is primarily driven by the potential involvement of omega-3 in the prevention and treatment in a number of inflammatory conditions (101). Omega-3 have been proposed to improve the structure of the cellular membrane, promoting cellular fluidity, whilst suppressing ROS and cytokine production (100). Jouris et al. (101) examined the effects of seven days of 3000 mg·day\(^{-1}\) of omega-3 consumption against 14 days of omega-3 dietary restriction prior to EIMD on a number of indices using a within subjects crossover design. Soreness was lower following supplementation, with no differences in arm volume or circumference and skin temperature. Similarly, Lembke et al. (102) examined ingestion of 2700 mg·day\(^{-1}\) of omega-3 against placebo for 30 days on strength indices, muscle soreness, indirect markers of inflammation and muscle damage following EIMD. Supplementation resulted in decreased soreness at 72 h, inflammatory markers which were lower at 24 h and no difference in other indices. Improvements in soreness were ascribed to improved concentrations of cellular markers indicative of cellular integrity leading to higher elasticity and flexibility and reduced primary damage following EIMD. The lack of improvement in functional capacity following supplementation observed in this study are supported by further research which has found no improvement in performance measures, despite improved markers of inflammation (117). Whilst omega-3 fatty acids may improve some markers of inflammation and soreness, the current research would suggest that this appears to have little bearing on improving recovery of functional capacity. Given the limited number of studies in this area, further research is needed to investigate omega-3 supplementation in EIMD models.

Curcumin is also thought to have potent anti-inflammatory effects and as such supplementation following EIMD has been investigated. Through its ability to scavenge free radicals, curcumin is purported to influence ROS production and may play a pivotal role in decreasing the production of pro-inflammatory cytokines and reducing the magnitude of the
inflammatory cascade (98). Using a novel delivery method to improve bioavailability, Drobnic et al. (99) examined 2 g·day$^{-1}$ of curcumin supplementation or placebo for two days prior to, and one day following EIMD on indices including soreness, MRI images of damage and indirect markers of inflammation. At 48 h following EIMD soreness and MRI evidence of muscle damage were lower in the supplementation group, whilst markers of inflammation were similar. Functional capacity indices were not investigated. A similar study examined supplementation of 150 mg of curcumin or placebo prior to and 12 h following EIMD on soreness, circumference, maximal voluntary contraction (MVC) and markers of muscle damage and inflammation in a crossover design (118). Markers of muscle damage and MVC improved significantly following supplementation compared to placebo. There were no significant differences between groups with respect to soreness or markers of inflammation. Using an animal EIMD model, Davis et al. (98) examined curcumin supplementation against control on time to fatigue and inflammatory markers following simulated downhill running. Compared to control, inflammatory cytokines and function were attenuated in the curcumin supplementation group at 24 and 48 h following EIMD. Taken together, the literature would suggest an inconsistent effect of curcumin on indices of EIMD. Improvements have been observed in markers of muscle damage and markers of functional capacity such as MVC and time to exhaustion whilst no change in other indices such as inflammatory markers have been observed. However, there is a paucity of research investigating curcumin in EIMD models and the relevance of these improvements to the ability to produce force or power and other functional capacity measurements is presently unclear.

A number of the aforementioned strategies have been implicated for providing benefit in recovery from EIMD. Polyphenol rich foods including consumption of berries and cherry juice supplementation have shown promise in aiding recovery following EIMD. Similarly, protein ingestion may assist in recovery by preserving intracellular proteins with benefits being most pronounced when the supplement contains up to 0.25 g·kg$^{-1}$ body mass of high quality dietary protein and is consumed following exercise. Given the equivocal nature of the current research and the potential for antioxidants such as Vitamin C and Vitamin E to negatively influence acute and chronic training adaptations, caution should be advised with consuming these supplements. Omega-3 fatty acid use appears to have limited application in promoting
recovery following EIMD, whilst supplementation with curcumin is inconsistent with select indices showing improvement and others showing no change. However, there is a paucity of research investigating these strategies and more research is needed to illustrate the effects of omega-3 and curcumin, in isolation or combination, on recovery following EIMD. The utility of these supplements in promoting recovery following EIMD may lie in being able to prescribe them in various combinations to ascertain if their effects compound.

2.2 IMPACT-INDUCED MUSCLE DAMAGE (IIMD)

2.2.1 AETIOLOGY OF IIMD

Muscle damage which occurs following competitive match play or simulations has been previously explored in the literature (3, 17). These models of muscle damage are likely to include both EIMD and IIMD, a form of muscle damage induced by compression of skeletal muscle by an external stimulus at or adjacent to the impact site (61). This insult results in direct myofibril damage, and in an attempt to regenerate the damaged tissue a cascade of primary and secondary damage associated cellular responses (25) including cellular necrosis, and invasion of pro-inflammatory factors (61, 119). Muscular damage following blunt-force trauma varies in severity from mild contusion through to large haematoma formation and to more clinically serious complications such as myositis ossificans, the proliferation of bone within the muscle (61). As contact-sport athletes engage in progressively more collisions as training or match duration continues, they are likely to be exposed to increases in IIMD. This may directly affect skill execution and performance during the match, and contribute to increased injury risk (120). Following IIMD, regeneration and the restoration of function typically occurs over an overlapping, tri-phasic model; damage, repair, and remodelling. These processes involve interactions between the immune and inflammatory systems and skeletal muscle (119). The systems interactions and two phase damage model are somewhat mirrored by those previously highlighted relating to EIMD, although EIMD is associated with accelerated recovery and a smaller magnitude of primary and secondary injury when compared to IIMD (61, 119). Whilst EIMD and IIMD have commonalities in regeneration and remodelling following injury, IIMD is divergent in the greater inflammatory infiltrate and subsequent secondary damage response (25). This divergence may provide the background
and rationale for the selection and application of interventions which can selectively mitigate or treat IIMD based on their mechanism of action.

Appropriate healing requires the coordinated action of a number of different local and systemic factors to facilitate muscular repair following IIMD. Following a mild contusion similar to IIMD, undamaged arterioles within the injured site dilate, increasing localised blood flow via two mechanisms. Firstly, histamines are released directly from mast cells present in the damaged area, which act to increase capillary permeability via enlargement of pores within the endothelial tissue (119). Similar to the response to EIMD, leucocyte number and concentrations of plasma proteins, both crucially involved in the inflammatory response, increase within the localised damaged tissue (41). Concurrently, inflammatory cells release prostaglandins which are thought to sensitise local nociceptors to induce the sensation of soreness (8). Secondly, local vasodilation is induced by the release of vascular endothelial growth factor (VEGF) which stimulates the NO pathway. VEGF can be secreted by a number of cells within the localised milieu including fibroblasts, macrophages and endothelial tissue (41). Neutrophils, which are immune cells produced from bone marrow cells, migrate to the damaged area via signalling from the aforementioned localised growth factors, such as cytokines. Similar in both IIMD and EIMD, neutrophil action is implicated in free radical generation during phagocytosis and secondary damage formation (41, 119). From the literature it is clear that complex interactions between local and systemic factors are needed to stimulate the healing and remodelling of skeletal muscle following IIMD.

It is worth noting that currently our understanding of the effects of impact trauma on skeletal muscle tissue is drawn from the field of experimental animal contusion (26). Typically these investigations have used a ‘drop-mass’ model, whereby a known mass is repeatedly dropped from a known height onto a stationary animal (typically rat) hind-limb (26). The histological response to this stimulus is then investigated and compared to pre-injury and/or control animal tissue. Often the force of the impacts are such that it produces changes in the underlying muscle tissue such as extensive myofibril necrosis, basal membrane disruption, underlying bone fracture and incomplete recovery, associated with severe muscle injury (26).
Histological and functional changes following contusion are likely to be injury severity-dependent (26). As such, the application of this research to contact-sport athletes may have merit, provided it can be manipulated to reflect the severity of the damage to which those athletes are habitually exposed (18), to ensure context validity.

2.2.2 EFFECTS OF IIMD ON PERFORMANCE AND RECOVERY

Human and animal research has been undertaken to investigate the purported effects of high intensity collisions and associated muscle damage on performance indices and neuromuscular recovery. IIMD can disrupt capillary networks, produce intramuscular bleeding, oedema, and inflammation (121, 122). These changes, along with associated rises in intramuscular pressure (61), have been linked with displacement of actin and myosin filaments and altered sarcomere function through decreased cross bridge formation during muscular contractions (122). Altered cross bridge formation may result in attenuated force generation capacity and this may provide the mechanism by which IIMD alters performance and the delay in neuromuscular recovery.

Preliminary research by Takarada (17) established the link between collisions and muscle damage. This study examined the relationship between tackles completed during match play and serum markers of muscle damage, [Mb] and [CK]. Large and significant associations were observed between the number of tackles completed and peak [Mb] \((r=0.852, P < 0.01)\), plus peak [CK] \((r=0.922, P < 0.01)\). These strong associations between physical contact interactions and muscle damage markers following contact-sport match play were further identified in a number of subsequent investigations (2, 3, 20, 24, 123). It is important to note that these studies examined collisions in the context of running based activity, which could have contributed to the extent of symptomology through EIMD. Similar relationships have been observed between the number of collisions and the change in lower limb rate of force development and peak power (18), plus subjective soreness (19) in professional RL populations. Taken together, these data suggest a potential link between IIMD and altered muscle damage markers, soreness and impaired athletic performance. However, beyond
correlational data, there is a dearth of research investigating the effects of IIMD on exercise capacity in a controlled manner.

There are a limited number of controlled studies investigating the effects of IIMD on performance and recovery in contact-sport populations. Studies have explored the effects of simulated collisions in the context of a small-sided game (21), intermittent-sprint protocol (124), and team-sport conditioning circuit (125) exercise protocols. These studies have shown significant decrements in performance measures including power and mean sprint time (21, 124, 125) following IIMD compared to non-impact control conditions. Despite this, an inconsistent response in the change in selected blood markers of muscle damage and subjective soreness has been noted following IIMD. Johnston et al. (21) identified significant increases in blood markers of muscle damage, and Singh et al. (125) observed increased soreness. However using similar markers and methodologies, others have observed no differences compared to control (124, 125). Differences in the intervention protocols and the severity of the collisions may explain this variance in response, with IIMD implemented via collisions with a tackle bag (125), lower body tackles performed by a research assistant (124), wrestling and shoulder pummels (21). These protocols may contain methodological limitations, including whether tackle bags simulate game related collisions in an ecologically valid manner (126). Additionally, the test-retest reliability of the use of tackle bags and tackling performed by a research assistant may be limited. Finally, the inclusion of wrestling and/or high intensity running in these studies increases the likelihood that EIMD may have resulted from the exercise protocols themselves. This may confound the results and limit the applicability of these findings in exploring the implications of IIMD on muscle damage and associated exercise performance. As such caution should be exercised when generalising based on these conclusions. To limit this, future research should aim to explore IIMD in the absence of EIMD and possible muscle damaging exercise.

From the animal research perspective, several studies have examined IIMD using a drop-mass model to study the subsequent symptomology (26, 122, 127-132). However, a limited number of these studies have investigated neuromuscular function following IIMD. Using a drop-mass
animal experimental model, Elmer et al. (122) explored the effects of repeated collisions on muscular force, work and power indices. They identified reductions in isometric force, power and submaximal work that were significantly reduced at 1 (28-33 %, p<0.05) and 48 h (28-38 %, p<0.01) following injury. Using a similar model, a recent investigation identified torque decrements that did not return to baseline until 14 days following injury (132). Kinematic analysis of animal gait following contusion injury to the lower limb revealed a similar pattern of recovery with recovery of identical angular positions during gait, and maximal range of motion to the non-injured limb at 14 days following injury (133). The attenuation in neuromuscular function was thought to occur due to inhibition of actomyosin cross bridge formation due to intracellular oedema, in addition to damage to the sarcoplasmic reticulum leading to inhibited calcium release and ultimately, alterations in ability to produce muscular work (122). These proposed mechanisms are consistent with the aetiology of force loss following EIMD (8, 28), potentially indicating a similarity in the extent to which secondary inflammation is a driving mechanism for performance changes following muscle damage.

Previous research in contact-sport populations has established the link between collisions, muscle damage and attenuated performance. Likewise, these studies have identified relationships between collisions and the rise in systemic muscle damage markers, further highlighting this link. Presently, the methodologies used to investigate this have suffered from methodological limitations which include questionable test-retest reliability and ecological validity. Furthermore, these methodologies have not been without the confounding influence of EIMD and thus caution should be exercised when examining and attempting to draw conclusions based on this research. Further research in the animal domain has highlighted the link between IIMD and altered neuromuscular function. Attenuated neuromuscular function is thought to occur in response to increased intracellular oedema resulting in inhibited cross bridge formation and damage to the sarcoplasmic reticulum modulating calcium release (122). While further research to establish the mechanism(s) in human populations is needed, investigations in this area may be limited by ethical considerations.
2.2.3 ADAPTATION TO IIMD

Researchers have postulated that an adaptation occurs within skeletal muscle from an initial bout of IIMD to any subsequent bout. This effect would be analogous with the repeated-bout effect observed in EIMD (35, 50). Presently, research is limited to a small number of studies with an effect yet to be equivocally established and as such a broader mechanism has yet to be proposed.

A number of studies have suggested that similar adaptation occurs following IIMD, termed ‘contact adaptation’ (134), although these suggestions are extrapolated from acute and longitudinal match play data and consensus does not exist. Hoffman et al. (135) were the first to propose an adaptation between IIMD and markers of muscle damage in a study investigating the performance and biochemical changes to a collegiate American Football match. No change was observed in systemic [CK] immediately following a match. They postulated that as the match was the final game in a 12 week regular season, an adaptation to IIMD occurred over the previous matches and training sessions, resulting in decreased muscle damage. An alternative hypothesis may be that there had been an adaptation that occurred similar to the repeated-bout effect to EIMD, as highlighted previously. However [Mb], another marker of muscle damage, was significantly elevated following the match compared to baseline. This seemingly inconsistent finding is potentially due to delayed kinetics of the larger CK molecules escaping damaged muscle tissue membranes into systemic circulation, in comparison to smaller Mb molecules. As such the peak of [CK] may have occurred beyond the testing time point and potentially missed in the period of data capture.

The same researchers examined changes in muscle damage markers in a longitudinal manner throughout a competitive season (136). Testing was undertaken at five time points including prior to and following the last day of the preseason training camp, and at weeks three, seven, and 10 of the competitive season. Testing for weeks three, seven, and 10 occurred at least 15 h following the previous training session. [CK] was found to be significantly elevated following the end of the preseason camp but similar to baseline at all other testing time points, whilst
[Mb] was similar to baseline at all testing time points. The authors speculated that the attenuation of [CK] following the initial training camp was evidence of adaptation to IIMD. Another potential explanation is that following a period of relative inactivity such as the off-season, when exposed to a period of increased training load such as a preseason camp, athletes were exposed to a degree of EIMD which resulted in increases in observed markers of muscle damage (136). This exposure produced a repeated-bout effect to EIMD which conveyed an adaptation observed to last the season. Interestingly, in a study investigating the longitudinal subjective soreness response to match play in elite RL, soreness was found to persist throughout the competitive season (19). Upper and lower body soreness was found to be similar across the phases of the competitive season. This data demonstrates that athletes experience similar subjective soreness regardless of phase of season and contests the idea of adaptation to IIMD.

The aforementioned research highlights the equivocal state of current research exploring adaptation to IIMD and further highlights the importance of investigating IIMD and the adaptive response in the absence from the potential confounding effects of EIMD. Furthermore, to truly investigate any adaptive response, the inclusion of performance-based metrics are necessary, which when combined with indices such as subjective soreness and markers of muscle damage, may provide a holistic model for investigating any adaptive response to IIMD. Similarly, studies exploring the relationship between these variables and the acute and longitudinal adaptive response to IIMD are limited. Finally, to effectively investigate adaptation to IIMD, athletes would need to limit their prior exposure to IIMD before undertaking research, to remove the potential influence on exposure and ensure they are not presenting having already adapted to IIMD. If the time course of adaptation is similar to that of EIMD, a period of six months or longer would be necessary to ensure such investigations are not influenced by this adaptation and therefore would be logistically difficult to undertake. Further investigations and exploration of adaptation to IIMD is clearly needed to define the existence, magnitude, and time course of any potential effect.
2.2.4 IIMD AND RECOVERY STRATEGIES

In contrast to EIMD, where a number of recovery strategies such as CWI, compression and dietary strategies such as antioxidants and protein supplementation have been explored, there are a limited number of studies which have examined strategies that may prevent injury and/or promote recovery from IIMD in human or animal models. These strategies have focused on modulating various aspects of the primary and secondary damage to limit the magnitude of damage and accelerate functional recovery. There are inherent differences between EIMD and IIMD in the magnitude of inflammation in the secondary damage (25), as such strategies that target this response such as antioxidants or CWI may be of particular benefit in aiding recovery from IIMD. Similarly, the use of protective equipment, which is commonplace among contact-sport athletes at all levels (137, 138), may be of benefit in decreasing the magnitude of primary damage to promote recovery of functional capacity.

Protective equipment

The use of prophylactic interventions like protective equipment has been evaluated from a number of perspectives including the prevention of injuries in a number of contact-sport cohorts. Research has identified that tackles and collisions accounting for over 50 % of injuries in adolescent RU athletes (139), with body contact the predominant cause of injuries in Australian Rules Football (140). Additionally, the lower-limb is the predominant site of injuries (35 %), with muscular and ligamentous injuries accounting for 65 % of all injuries in RU athletes (141). An investigation by Marshall et al. (137) investigated the efficacy of protective equipment in preventing injury in recreational level RU athletes and identified that the use of padded headgear and support sleeves reduced soft-tissue injuries, such as contusions, strains and sprains. Other strategies such as strapping, taping, and guards were not effective in reducing injury. Similarly, Mitchell (142) investigated the effectiveness of thigh protectors in reducing contusion and haematoma in junior Australian Rules Football. They identified that wearing thigh guards reduced the number of haematomas experienced throughout a season, indicating that protective equipment may play a role of reducing the symptoms of muscle damage caused by an external stimulus (142). Whilst these studies did not examine the effect of prevention strategies on IIMD per se, this research does highlight the prevalence of
muscular injuries and significance of collisions to these injuries. Similarly, this research highlights the widespread use of protective equipment as an effective prevention strategy. If a model of IIMD could be established, the efficacy of these strategies on preventing IIMD could then be explored.

Cryotherapy

In an effort to potentially limit the inflammatory response, and the associated secondary damage, suppressing the neutrophil response and associated free radical production would theoretically be a target area. Indeed, animal investigations have found recovery strategies such as cryotherapy can attenuate inflammatory cell infiltration and modulate oedema (143). However, research has identified conflicting functional effects as the application of topical ice delayed regeneration and resulted in denser scar tissue formation following injury (143). Despite cryotherapy strategies (such as topical ice) being a commonly used treatment for muscular contusion, there is little experimental evidence for its efficacy in promoting muscle recovery in human studies (62). Given this lack of human data and potential for cryotherapy to delay functional recovery, caution is advised in the use of cryotherapy as a target treatment for IIMD (143).

Therapeutic ultrasound

Therapeutic ultrasound has been investigated in animal contusion research models as a strategy to promote recovery via increased blood flow leading to reduced oedema (88, 89). The therapeutic potential of ultrasound following IIMD was initially confirmed by research which found increased satellite cell proliferation following ultrasound exposure subsequent to contusion injury (131). However, in a recent study using a similar model, daily ultrasound exposure was found to have no effect on healing of muscle tissue or muscle nuclei number (88). Similarly, when ultrasound was combined with exercise, no benefit was observed following contusion injury (89). These studies highlight the current lack of evidence in the utility of therapeutic ultrasound as a treatment strategy for IIMD in animal models, with no studies in human populations.
Dietary strategies

Dietary strategies such as polyphenol supplementation have been examined for a potential role in promoting recovery the animal contusion context. Given the major role of ROS in IIMD (119), supplementing with antioxidants, such as polyphenols, as a treatment strategy to augment repair and promote recovery would seem pertinent. Indeed, research has investigated supplementation with antioxidant-rich grape seed derived polyphenols following contusion injury (130). Supplementation lead to an improved satellite cell response, decreased neutrophil infiltration, and decreased inflammatory cell response following contusion, suggesting an accelerated recovery following supplementation (130). Follow up investigations by these researchers confirmed these findings (144), and identified that polyphenol supplementation accelerated the rate at which invading macrophages switched from a pro-inflammatory to an anti-inflammatory phenotype (129). Collectively, these studies highlight a potential supporting role for foods rich in polyphenols in facilitating recovery. These findings are compounded by the previously highlighted evidence supporting recovery with foods rich in antioxidant’s following EIMD, the potential role of these foods following IIMD is worth exploring.

Other supplementation strategies such as omega-3 polyunsaturated fatty acids have shown promise for augmenting the inflammatory response following exercise (100) and the antioxidant systems of skeletal muscle cells in vitro (145). Similarly, curcumin has been shown to exhibit anti-oxidant and anti-inflammatory properties (146) and attenuate inflammation following EIMD (98). Whilst these findings are important, research did not examine if supplementation and improvement in the inflammatory response was associated with an improved recovery of indices of neuromuscular function following IIMD. As such, it is difficult to draw conclusions based on the limited data. However, given that these strategies target the inflammatory response, they may be suited for use prior to and following IIMD as a treatment strategy. Further research in animal and human models is needed to establish if polyphenols and/or other dietary strategies, which have shown promise in EIMD models (92),
have efficacy in reducing the inflammatory response and in promoting functional recovery following IIMD.

It is important to note that when considering the efficacy of strategies that target the inflammatory response, a recent review has highlighted that interfering with the kinetics of inflammation negatively alters recovery from muscular injury (29). The use of strategies such as non-steroidal anti-inflammatory drugs (NSAIDs) and cryotherapy were associated with greater collagen deposition, decreased macrophage infiltration, and smaller regenerated muscle fibres following recovery (29). This research underlines the crucial role of the acute inflammatory phase following injury in signalling subsequent sequences of regeneration. As such caution should be advised when prescribing anti-inflammatory strategies following IIMD. Given this finding and the current lack of evidence for the utility of these strategies, more research is necessary to establish if these strategies improve recovery following IIMD.

Future directions

As previously discussed, IIMD is associated with a greater magnitude in secondary inflammatory damage when compared to EIMD. Given this, strategies which are predicated on targeting the inflammatory response, such as compression garments and CWI may have potential in accelerating recovery following IIMD. Compression garments are thought to decrease pain via creation of an external pressure gradient thereby reducing the space for oedema to occur and causing a decrease in sensitized nociceptors (55). Furthermore, garments are thought to reduce markers of muscle damage via improved circulation from enhanced muscle-pump function, although this remains speculative (55). Similarly, CWI may attenuate pain via vasoconstriction, reduced perfusion, and decreased local capillary permeability reducing oedema, and in turn the pressure applied to type IV afferent nociceptors (78). Likewise, the reduction in temperature has been proposed to limit ischemic damage to uninjured tissue via these pathways (78). As such, these strategies may hold benefit for reducing oedema and promoting recovery following IIMD, and should therefore be explored as an area of further research.
At present there is a dearth of literature examining strategies which may attenuate IIMD. Indeed, a recent review has highlighted the limited evidence of adjunct treatment strategies (147). Given the lack of evidence for effective strategies and possibly due to the potential for particular strategies to hinder the adaptive response, they recommended conservative treatment such as mobilisation and progressive, graduated loading of the damaged tissue in promoting successful functional recovery following muscle injury (147). However, these recommendations would appear to be aimed at athletes who have a serious muscular injury (such as a strain), and not athletes looking to accelerate recovery from IIMD over a much shorter time frame, such as that between matches. As such it is important for researchers and practitioners to understand the context in which these strategies are investigated and applied. Similarly, given the potential for strategies to negatively influence long-term muscular recovery, applying an intervention acutely, for example when duration between sessions or exercise bouts is limited, and not in a chronic manner is presently recommended.

The current absence of studies investigating IIMD in contact-sport populations is primarily driven by ethical considerations and participant recruitment factors regarding exposing participants to inappropriate risk of injury. Establishing an appropriate and safe intervention by which to apply IIMD is needed to allow exploration and identification of recovery strategies that may attenuate the recovery timeline in contact-sport populations. Given that they specifically target aspects of the secondary, inflammatory response, CWI, compression, and polyphenol supplementation may be strategies of interest in future research examining promoting acute recovery following IIMD.

2.3 SUMMARY

Athletes participating in contact-sports are regularly exposed to muscle damage due to the demands of competition and training. This muscle damage can be categorised as impact-induced or exercise-induced, with IIMD being comparatively unexplored. The tissue response to IIMD occurs through three overlapping stages; damage, repair and remodelling. These processes involve multifaceted interactions between the immune and inflammatory systems, specific cytokines, and growth factors. There are similarities between EIMD and IIMD in the
phases of progression of the primary and secondary damage response, and the induction of factors such as leukocytes, macrophages and ROS, crucial for optimal muscle fibre recovery. The divergence between EIMD and IIMD comes from the initial damage stimulus, in the greater magnitude of primary and secondary damage, and the delayed progression of the secondary, inflammatory damage following IIMD (28). Given these differences, there is potential for an independent timeframe to exist for effects on performance and subsequent recovery following IIMD in contact-sport athletes. Similarly, there is potential for interventions to preferentially target IIMD based on the interventions mechanism of action and the mechanism(s) associated with IIMD. Currently, human data is limited with observations typically drawn from animal experimental research, whereby a drop-mass model is used to investigate impact trauma on skeletal muscle tissue. In these animal models changes including tissue necrosis, bone fracture and incomplete functional recovery are observed, with changes appearing to be damage severity dependent. Given the scarcity of human and athlete data, and the observation that athletes typically recover during their microcycles (18, 148), caution should be observed when extrapolating these findings to contact-sport athletes. Further research is needed to explore this gap in understanding to ensure that the findings in humans can be evidence-based.

Initial research in contact-sport athletes has established strong relationships between collisions, markers of muscle damage and performance impairment. Further controlled research has investigated IIMD in the context of exercise and identified attenuated neuromuscular function and athletic performance in the days following IIMD. Given the previously highlighted limitations of this research, the performance implications of these alterations in neuromuscular function remain to be elucidated. Animal research exploring changes in performance has identified decrements in the ability to produce force and power in the hours and days following IIMD. Alterations in the ability to produce force in human and animal models are postulated to be mediated through inhibition of cross bridge formation and muscle fibre damage secondary to altered calcium flux. However, currently there is a lack of evidence from human models to determine if this hypothesis is accurate, and highlight the extent of implications on functional capacity following IIMD.
Current controlled studies using contact-sport athletes have investigated IIMD in the context of high-intensity exercise. From an ecological validity perspective, this may appear as a reasonable approach. However, high-intensity and/or eccentric exercise is likely to result in EIMD which may lead to underlying changes in indices of recovery. This may influence these data and potentially confound results and conclusions. Therefore to understand the magnitude and time course of IIMD, investigations are needed which examine IIMD in the absence of high intensity and/or eccentric exercise. The establishment of a model of IIMD in lieu of EIMD would afford investigators an opportunity to explore the performance implications and associated timeline of changes following IIMD. Similarly, establishment of a model would allow investigators to explore adaptation to IIMD, as has been discussed in the literature (19, 135, 136). Furthermore, this model could be used to identify interventions and strategies that have efficacy in mitigating, treating, and managing IIMD.
Chapter 3. IIMD: Performance Implications and Associated Timeline

3.1 ABSTRACT

PURPOSE: The implications of impact-induced muscle damage (IIMD) that results from participation in contact-sport are not well understood. The purpose of the present study was to implement a systematic and novel method of generating IIMD in the absence of exercise-induced muscle damage (EIMD) and characterise the implications of this on an array of parameters, including muscle damage and exercise performance.

STUDY DESIGN: Single-group experimental time-series with repeated measures.

METHODS: Eighteen male recreational contact-sport athletes completed a single-group time series with measures assessed at baseline (PRE) and immediately following (POST) an IIMD protocol, with repeat testing 24, 48, and 72 h following the IIMD protocol. Biochemical indices of muscle damage (myoglobin [Mb]) and inflammation (high-sensitivity C-reactive protein [hs-CRP]), ultrasound assessment of oedema, 15 m sprint performance, squat jump peak power (SJ-PP), perceived mood, subjective function and soreness were compared to PRE measures using a one-way (time) repeated measures ANOVA with post-hoc student t tests.

RESULTS: Speed over 5, 10, and 15 m were impaired for 48 h (7.5 ± 4 %, \( P < 0.01 \)) and SJ-PP was impaired for 48 h following the IIMD protocol (9.5 ± 3 %, \( P < 0.01 \)). IIMD significantly altered perceived function for 24 h (76 ± 4 A.U. vs 73 ± 3.5 A.U., \( P < 0.01 \)) and perceived mood for 48 h (0 ± 1.8 A.U. vs. 3 ± 3.1 A.U., \( P < 0.01 \)). Subjective soreness was elevated from baseline for 72 h (\( P < 0.01 \)) following the IIMD protocol. No change in ultrasound-derived oedema, [CRP] or [Mb] was observed (\( P > 0.01 \)). Strong correlations were observed between change in soreness measures and SJ-PP at 48 (\( r = 0.514 - 0.538, \ P < 0.05 \)) and 72 h (\( r = 0.497 - 0.594, \ P < 0.05 \)).

CONCLUSION: For the first time, the present research has shown that IIMD generated in the absence of exercise resulted in impaired performance including the ability to produce power and speed. Similarly, IIMD negatively influenced perceived mood, function, and subjective
soreness. These changes were most pronounced in the 48 h following the IIMD protocol. No change in muscle damage or inflammation indices were observed following IIMD, primarily due to the highly variable response. The experimental protocol used in the present study was successful in inducing IIMD in the absence of EIMD and therefore may be used as a model to further investigate other aspects of IIMD.

3.2 INTRODUCTION

Sports such as Rugby League (RL) and Union (RU) are characterised by intermittent periods of high-intensity movement such as sprinting, jumping, and tackling, separated by periods of relatively low-intensity activity such as standing, walking, and jogging (149, 150). In elite RL match play, athletes are required to run at 90-100 m·min$^{-1}$ and execute repeated high-intensity efforts, previously defined as $\geq$3 high acceleration ($\geq$2.79 m·s$^{-2}$), high speed (5 m·s$^{-1}$), or contact efforts with less than 21 s recovery between efforts (151). It has previously been shown that this cumulative physical work has the potential to delay recovery of neuromuscular function by up to 48 h following match play (148). This delay in athletic recovery can be partly attributed to a rise in exercise induced muscle damage (EIMD), referring to muscle damage typically occurring from unaccustomed or unfamiliar exercise (39). Often this occurs in response to movements that involve repetitive eccentric or high-intensity contractions such as plyometric exercise (14), wrestling (15), and repeated sprints (13) and can cause disruption to sarcomere integrity (46), elevate blood markers of muscle damage and inflammation (56), and impair the ability to produce force/torque (40). In addition, EIMD often leads to an increase in subjective pain following a latency period which typically peaks 24-48 h following injury (39), a phenomenon known as delayed onset muscle soreness (DOMS) (46).

In addition to the aforementioned physical demands and muscle damaging exercise, rugby athletes frequently engage in collisions with opponents, team mates and the playing surface. These collisions are sport specific. For example, elite RL players were found to physically collide 24-47 times per game, depending on position (152), whilst $\sim$14-44 collisions per game have been observed in elite RU (3). These collisions have been found to lead to decrements
in performance (18) and a rise in markers of muscle damage following match play (2, 3, 17). Moreover, collisions have been linked to soreness experienced in the hours and days following match play, that may persist throughout a competitive season (19). These data suggest skeletal muscle ultrastructural damage results from blunt force trauma and may profoundly delay recovery. However, match play data provides insight into changes related to accumulated neuromuscular fatigue, and the aforementioned parameters are often used as criteria to evaluate individual changes in response to both EIMD and impact induced muscle damage (IIMD) (2). Therefore, the possibility of conflation of the effects of EIMD and IIMD exists. As such, the magnitude and time course of alterations in neuromuscular function and performance specifically resulting from IIMD remains to be elucidated. Identification of these potential changes may provide insight into the potential implications and subsequent management of IIMD.

Research investigating the physiological effects and associated functional implications of IIMD in a controlled manner is limited. To date, studies have explored the effects of additional physical collisions in the context of a small-sided game (21), intermittent-sprint protocol (124), and a team-sport conditioning circuit (125). These investigations have identified a significant attenuation in performance, and increases in indices of muscle damage, when compared to a non-contact condition. Collectively, these data suggest that the addition of physical collisions to exercise results in IIMD (2), an exacerbated decrement in muscle function, and consequently performance. However, the inclusion of high-intensity running and/or wrestling in these studies introduces eccentric muscle action and associated EIMD as a possible source of such changes (13, 15). These exercise modalities have the potential to cause EIMD, and may confound any conclusions specifically relating to the implications of IIMD. As such, whilst these studies have merit in examining impacts in somewhat of an ecologically valid manner, in the context of exploring IIMD considerable limitations exist. In an effort to minimise this, research exploring IIMD should aim to do so in the absence of EIMD and possible muscle damaging exercise.
While there is lack of a repeatable experimental protocol with which to examine IIMD in the absence of EIMD in humans, the experimental animal literature has described a method for generating IIMD, using a ‘drop-mass’ model (26). Briefly, this model describes a method used previously for the release of a known mass from a known height onto an animal’s stationary lower-limb, typically to investigate recovery with respect to histological, performance, and kinematic changes (26, 129, 132). This model has been used to investigate tissue damage across various domains from minor muscular contusion to extensive tissue necrosis, underlying bone fracture, and incomplete recovery (26). Modification of such a method to contact-sport collision characteristics would provide a repeatable intervention with which to study IIMD in isolation and its associated effects. Therefore, the purpose of the present study was to capture and characterise the typical magnitude and duration of changes resulting from a standardised IIMD experimental protocol somewhat simulating collisions experienced during rugby match-play in athletes who are habitually exposed to blunt force trauma. The IIMD protocol was adapted to this population by exposing participants to a similar frequency and intensity of collisions, derived from published match-play research (27), to which they typically experience.

3.3 METHODS

Participants

Eighteen young, healthy men (Table 1) who regularly participate in contact sports such as RL or RU volunteered to participate in the present study. Participants had no recent history of lower-limb muscle, joint, bone or blood-related injury or health issue and provided written informed consent. The present study was approved by the University of the Sunshine Coast Human Research Ethics Committee. Participants were required not to perform any unaccustomed exercise or vigorous physical activity for the two weeks prior to testing and for the duration of participation. Similarly, participants were instructed not to use anti-inflammatory medication or engage in recovery strategies such as cold water immersion or compression garment wear for the duration of the study. Finally, testing occurred during the off-season to ensure participants were not exposed to additional game or training related fatigue or muscle damage. Sample size was estimated using a validated web-based power and
sample-size estimating program (GLIMMPSE, http://www.glimmpse.com), which features determining sample size for repeated measures studies (153). The sample size was based on data, including mean and variance from two previous related studies (21, 125) and based on the effect size of 1, alpha level of 0.05 and power (1-β) of 0.80. Using this information, a sample size of 18 was determined as necessary to ensure the study was appropriately powered.

Table 1. Descriptive characteristics including dual energy x-ray absorptiometry (DEXA) determined body composition data of the 18 participants (mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.8 ± 4.4</td>
<td>19 - 32</td>
</tr>
<tr>
<td>Stretch stature (cm)</td>
<td>180.0 ± 7.9</td>
<td>164.5 - 189.7</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>85.3 ± 10.4</td>
<td>68.0 - 105.8</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>16.4 ± 5.8</td>
<td>7.3 - 30.9</td>
</tr>
<tr>
<td>Lean tissue mass (kg)</td>
<td>65.3 ± 9.3</td>
<td>52.6 - 88.7</td>
</tr>
<tr>
<td>Bone mineral content (kg)</td>
<td>3.6 ± 0.4</td>
<td>2.6 - 4.4</td>
</tr>
</tbody>
</table>

*Experimental protocol*

Participants were familiarised with testing procedures at least two weeks prior to testing (75). During familiarisation, participants were familiarised with the questionnaire and all testing equipment and procedures, including the completion of four maximal 15 m sprints and four submaximal squat jumps separated by a 3 min recovery period. Participants were visually familiarised with the impact simulating equipment, however, no impacts were implemented prior to testing to ensure no muscle damage was introduced that may have influenced the study outcomes.
In the week prior to testing participants undertook Dual Energy X-ray Absorptiometry (DXA) to assess body composition. At the commencement of each testing session, participants had a venous blood sample collected for analysis of biochemical parameters. Thereafter, ultrasound images were collected, 15 m sprint and squat jump performance assessed, and finally a recovery questionnaire administered to assess subjective mood state, perceived lower-limb function, and perceived soreness. Following completion of baseline measures, the IIMD protocol was implemented. The same biochemical, ultrasound, performance and subjective measures were replicated immediately after the IIMD protocol, and again at 24, 48, and 72 h thereafter.

Dual energy X-ray absorptiometry (DXA)

Prior to undertaking the IIMD protocol, participants completed body composition assessment using DXA (Lunar iDXA, GE Healthcare, UK) fan-beam scan undertaken with current best-practice methodology (154). Participants were overnight fasted and free from exercise on the morning of assessment. Body mass was measured to the nearest 0.1 kg on electronic scales (SECA GmBH, Germany) and stretch stature to the nearest 0.01 m on a wall-mounted stadiometer (Harpenden, Holtain Limited, Crymych, United Kingdom), using previously described protocols (155).

Total body bone mineral density, fat mass and lean tissue mass were assessed. The DXA was calibrated using phantoms as per the manufacturer’s guidelines on each testing day prior to measurement. Participants wore minimal clothing and were positioned centrally on the scanning bed with foam hand and feet positioning aids. All tests were conducted by the same experienced and licenced operator. The scans were analysed automatically using GE enCORE v.13 software (GE Healthcare, UK) with the Geelong reference database and regions of interest subsequently confirmed by the operator. Whole body composition data was included for analysis.
Blood markers

Venous blood samples were collected at the beginning of each testing time-point. Each sample (5-8 mL) was collected from the superficial antecubital vein using standard venepuncture techniques. All samples were collected into serum separator tubes (SSI, Victoria, Australia) allowed to clot, and the serum separated at 1200 rcf (centrifugal force) for 10 min. Each serum sample was frozen and stored at -20 °C until analysis for concentrations of Myoglobin ([Mb]) and high-sensitivity C-reactive protein ([hs-CRP]). Concentrations of [Mb] were determined using an Integra 800 (Roche Diagnostics, NSW, Australia) immunoturbidimetric assay method (156). Concentrations of [hs-CRP] were determined using an Abbott C16000 (Abbott Diagnostics, CA, USA) immunoturbidimetric technique with commercially available assay kits (157). All samples were analysed by Douglass Hanly Moir commercial pathology laboratory (Macquarie Park, NSW, Australia).

Ultrasonography

Immediately following venous blood sample collection, B-mode ultrasound images were obtained from the right thigh markings of participants to quantify the inflammatory response within the muscle tissue (158, 159). The participant’s superior border of the patella was identified and using a surgical pen a reference landmark made 15 cm proximal to this (DIST), with a second landmark made 10 cm proximal from the initial position (PROX) (Figure 1). Images were obtained using a Terason t3000 Ultrasound System (Terason, Burlington, MA, USA) with a 7.5 MHz linear probe attachment interfaced with a PC. The probe was placed transversely at the impact sites, whilst the participant lay prone on the scanning bed. The investigator maintained adequate gel between the skin and probe, and applied limited pressure throughout testing. The gain, contrast, and time-gain compression settings were standardised and maintained throughout the testing period (159). Images were saved in TFF format and analysed by a specialised freeware image analysis program (ImageJ, Bethesda, MD, USA) (160). The region of interest was set as the muscle tissue immediately below the superficial subcutaneous adipose tissue and within the visible facial boundaries. The average echo intensity based on a histogram of grey scale (0: black, 256: white) for the region of interest was calculated. A shift towards a whiter (lighter) echo intensity would indicate the
presence of oedema consistent with the invasion of inflammatory fluid within the muscle (158, 161). The relative change in echo intensity from pre-exercise was recorded in duplicate for each image and the average of these values accepted for subsequent analysis.

![Representative reference markings for the locations of ultrasound images captured at the anterior distal (DIST) and proximal (PROX) thigh.](image)

Figure 1. Representative reference markings for the locations of ultrasound images captured at the anterior distal (DIST) and proximal (PROX) thigh.

**Performance Measures**

**15 m sprint**

Following ultrasound assessment, participants completed a standardised warm-up consisting of a 5 x 30 m jog, during which participants were instructed to maintain a low intensity (2/10) on Borg’s CR10 rating of perceived exertion (RPE) scale (162). Participants were then required to complete two 15 m sprints, separated by 2 min recovery, on a tartan running track. Dual-beam timing gates (SMARTSPEED, Fusion Sport, AUS) were placed at 0, 5, 10, and 15 m to accurately record split times (s). The mean of two attempts was recorded for statistical analysis. Participants were instructed to begin within 1 m of the initial timing gate and sprint as explosively as possible. Verbal encouragement was given by the investigator throughout
testing. Using a similar timing-gate system the test-retest reliability of sprinting with rugby athletes has shown to be appropriate, with less than 5% coefficient of variability (CV) (163).

**Squat jump (peak power)**

Following completion of the 15 m sprints, participants were required to complete two squat jumps (separated by 3 min) using a 20 kg standard Olympic bar. This load was chosen as it has been shown that when compared to greater loads such as 40, 60, and 80 kg, it is the load which elicits the highest peak power (164). Additionally, given the potential range of participants previous lifting experience, using greater loads may have proven unsafe and potentially introduced muscle damage. The mean of two attempts was recorded for analysis. Participants were instructed to lower the bar to a 90° knee flexion position, pause for 2 s, and then jump upward as explosively as possible (75). Squat jump peak power (SJ-PP; Watts (W)) was calculated using a GymAware linear position transducer (Kinetic Performance, AUS) interfaced with an iPod (Apple, CA, USA). Data derived from linear position transducers has been found to be both valid and reliable in the measurement of jump variables (165).

**Recovery questionnaire**

A questionnaire was developed to assess subjective recovery. Participants were required to complete this questionnaire at each testing time point. The Brief Assessment of Mood (BAM) required participants to rate their mood based on six factors (anger, tension, depression, vigor, fatigue, and confusion) of the Profile of Mood States (POMS) questionnaire (166) that has previously been used to monitor neuromuscular fatigue (22, 23). Participants gave a rating on a 5-point Likert scale anchored by one (not at all) and five (extremely). Mood disturbance (BAM-MD) scores were calculated as the vigor score subtracted from the total of the five other mood disturbances, with higher scores indicating greater mood disturbance (22).

The Lower Extremity Functional Scale (LEFS) was used to rate each participants ability to complete a variety of tasks as a result of perceived dysfunction (167). Participants gave a
rating anchored by zero (extremely difficult or unable to perform) to four (no difficulty) on a variety of activities of varying difficulty including walking, squatting, running, sitting and hopping. An overall score was then calculated based on a total of 80 points, with lower scores indicating greater perceived dysfunction, nine points being the minimal level of detectable change (167). This scale has been found to be reliable and have appropriate construct validity (167).

Finally, the Visual Analogue Scales (VAS) was used to monitor changes in subjective soreness and pain (8). Participants marked with a vertical line on a scale from zero (no pain) to 100 mm (worst pain imaginable) and completed a VAS for pain and discomfort at passive rest (VAS-PAS), following completion of three bench step-ups (VAS-ACT), and after palpation of the impact sites (VAS-PALP).

**IIMD Protocol**

A ‘drop-mass’ model based on animal experimental research was adapted for use in the present study (26). A Smith Machine was modified to include a flat 35 x 10 cm metallic impact surface which was covered in 4 cm of rubber to mimic the density of human skeletal muscle tissue (~1.1 g·cm³) (168) (Figure 2).
Figure 2. The rubber padding designed to mimic the density of skeletal muscle tissue affixed to a metallic impact surface used in the impact induced muscle damage (IIMD) collision simulator.

On arrival, the DIST landmark was extended parallel to the axis of the ground from the initial anterior thigh position through the lateral and posterior thigh. Similarly, the PROX landmark was extended parallel to the axis of the ground and through the lateral and posterior thigh (Figure 3).
The total system mass (bar and load) was 80 kg, a load consistent with reported body mass (kg) data from athletes of a similar recreational playing level (169). In order to replicate the frequency of impacts observed amongst semi-professional RL athletes (27), the total frequency of impacts was set at 26 over 80 min (~0.33 impacts·min\(^{-1}\)). This frequency was determined based on piloting data in which participants were gradually exposed to additional impacts and their responses recorded. Based on these data, the frequency and intensity of impacts was determined to be safe. Participants rotated through landmarks in a cyclic manner from anterior (DIST then PROX), to lateral (DIST then PROX) and finally posterior (DIST then PROX), until the total of 26 impacts had been completed. There were no adverse injurious outcomes to the testing procedures during the study and participants did not report any undue soreness or pain during testing.

At the completion of all baseline testing procedures, participants were placed in a seated position below the Smith Machine impact surface, with knees at 90° flexion, below the DIST landmark (Figure 4a). The bar was situated transversely and was lowered to a position ~25
cm above the DIST landmark, the impact surface was then released to impact on this location. The bar was reset and participants were instructed they could move freely, if necessary. Participants were then seated below the PROX landmark and the identical protocol completed. For lateral impacts, participants were instructed to lie on their side, with padding provided to minimise knee collisions (Figure 4b). For posterior impacts, participants lay prone on a standard bench at each of the impact landmarks (Figure 4c). Following completion of 26 total impacts, the aforementioned measures were assessed (POST), with testing replicated at 24, 48, and 72 h thereafter.

![Figure 4](image)

Figure 4. Representative positioning of the participants for the a) anterior impacts, b) lateral impacts, and c) posterior impacts during the impact induced muscle damage (IIMD) protocol.

### 3.4 STATISTICAL ANALYSIS

Data are reported as mean ± standard deviation (SD). To assess changes in each dependent variable following the experimental protocol, repeated measures one-way analysis of variance (ANOVA) were utilized. Mauchly’s test was used to establish sphericity and on identification of a violation of sphericity, the Greenhouse-Geiser correction was applied. For the repeated measures ANOVA, Mauchly’s test of sphericity indicated that [hs-CRP], [Mb], PROX-GSA, 10 and 15 m sprint split times, BAM-MD, LEFS, VAS-PASS, VAS-ACT and VAS-PALP output violated the assumption of non-sphericity and as such significant main effects for these variables were assessed using Greenhouse-Gessier corrections.
On identification of a significant $F$-ratio, paired sample $t$-tests were undertaken with Bonferroni adjustment for multiple comparisons to determine statistical significance difference compared to PRE values. Significance was set at $P \leq 0.01$ due to multiple comparisons. A mean substitution method was employed when missing data values occurred between two available values, and participant data was excluded when missing data occurred at either baseline or at 72 h follow-up. Standardised residuals were calculated from each observation and a value greater than 3.0 was considered to be an outlier, and removed from analysis. Due to missing data and the presence of outliers, 16 complete datasets were included for Ultrasound analysis and 17 for [hs-CRP] analysis. Statistical analysis was undertaken in SPSS version 17 (IBM, USA). The change in selected dependent variables compared to baseline was undertaken using Pearson product-moment correlation coefficients. Raw data for performance scores is available (Appendix 1).

### 3.5 RESULTS

**Biochemical markers**

No significant main effects for time were observed for either [hs-CRP] or [Mb] ($P > 0.01$).

**Table 2.** Results (mean ± SD) of biochemical markers of inflammation (high-sensitivity C-reactive protein [hs-CRP]) and muscle damage (Myoglobin [Mb]) including probability ($P$). $n = 17$ complete datasets included for [hs-CRP] analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>[hs-CRP] (mg/L)</td>
<td>1.1 ± 1.0</td>
<td>2.6 ± 3.2</td>
</tr>
<tr>
<td>[Mb] (ng/mL)</td>
<td>35.9 ± 15.4</td>
<td>46.5 ± 20.4</td>
</tr>
</tbody>
</table>
Ultrasonography

No significant main effect for time for either DIST-GSA or PROX-GSA was observed (DIST-GSA: $F(4,60) = 0.897, P = 0.472$; PROX-GSA: $F(2.68,40.27) = 1.151, P = 0.337$) (Figure 5).

![Figure 5](image.png)

**Figure 5.** Percent change in grayscale analysis of ultrasound images taken at the anterior distal (DIST-GSA) and proximal (PROX-GSA) impact sites (arbitrary units (A.U.)) (mean ± SD; n = 16 complete datasets). Data represents mean grayscale analysis of ultrasound scans of the anterior impact sites expressed as a percentage of baseline values.

Sprints

ANOVA revealed a significant main effect for time for speed over 5, 10 and 15 m (5 m: $F(4,68) = 21.382, P < 0.001$; 10 m: $F(2.41,40.9) = 19.987, P < 0.001$; 15 m: $F(1.99,33.83) = 20.547, P < 0.001$). When compared to PRE, the percentage change in 5, 10, and 15 m split times were significantly increased immediately following (POST), and at 24 and 48 h, but not at 72 h following the IIMD protocol (Figure 6).

The percentage change at POST was significantly greater for sprint velocity at 5 m when compared to 10 ($P < 0.001$), and 15 m ($P < 0.001$) (Figure 6). Similarly, at 24 h, 5 m sprint
velocity percentage change from PRE was significantly higher than for 10 ($P = 0.008$) and 15 m ($P = 0.006$). There were no significant differences at any other time points ($P \geq 0.052$).

![Graph showing sprint times](image)

**Figure 6.** Percent change in 15 m sprint time before, immediately after and 24, 48 and 72 h following impact induced muscle damage (IIMD). Data represents 5, 10, and 15 m split times expressed as a percentage of PRE values (mean ± SD). *Significant ($P < 0.01$) difference from PRE. # Significant ($P < 0.01$) difference to 10 and 15 m.

**Squat Jump (peak power)**

Squat jump ANOVA analysis revealed a significant main effect for time ($F(4,68) = 41.621$, $P < 0.001$). The percentage change in peak power was significantly reduced immediately following the IIMD protocol ($P < 0.001$), at 24 ($P < 0.001$), and 48 h post-IIMD ($P = 0.004$), with no difference at 72 h post-IIMD ($P = 0.800$) (Figure 7).
**Figure 7.** Squat jump peak power (SJ-PP) expressed as a percentage of PRE values (mean ± SD) before (PRE), immediately after (POST), 24, 48 and 72 h following the impact induced muscle damage (IIMD) protocol. *Significant ($P < 0.01$) difference from PRE.

**Recovery questionnaire**

Statistical analysis revealed significant main effect for time and BAM-MD ($F(2.13,36.25) = 10.83, P < 0.001$). When compared to PRE, BAM-MD was significantly elevated POST ($P < 0.001$), 24 h ($P = 0.001$) and 48 h ($P < 0.001$) following the IIMD protocol (Figure 8). No significant difference was observed 72 h post-IIMD ($P = 1.00$).
Figure 8. Change in Brief Assessment of Mood – Mood Disturbance (BAM-MD) (A.U.) over testing time points (mean ± SD) before, immediately after and 24, 48 and 72 h following the impact induced muscle damage (IIMD) protocol. *Significant ($P < 0.01$) difference from PRE.

Statistical analysis of LEFS revealed a significant main effect for time ($F(2.251,38.274) = 6.644, P = 0.002$). When compared to PRE, LEFS was significantly decreased POST ($P < 0.001$) and 24 ($P = 0.005$) (Figure 9). No significant differences were observed 48 ($P = 0.088$) or 72 h ($P = 0.564$) following the IIMD protocol.
Figure 9. Change in Lower Extremity Functional Scale (LEFS) score (0-80) (A.U.) before, immediately after and 24, 48 and 72 h following the impact induced muscle damage (IIMD) protocol (mean ± SD). * Significant \( (P < 0.01) \) difference from PRE.

Analysis of VAS-PASS, VAS-ACT, and VAS-PALP revealed significant main effects for time (VAS-PASS: \( F(2.46,41.77) = 17.581, P < 0.001 \); VAS-ACT: \( F(2.05,34.8) = 21.894, P < 0.001 \); VAS-PALP: \( F(2.32,39.45) = 35.21, P < 0.001 \)). Compared to PRE, VAS-PASS was significantly elevated immediately following the IIMD protocol \( (P = 0.001) \) (Figure 10). Similarly, when compared to PRE, VAS-PASS was elevated 24 \( (P < 0.001) \), 48 \( (P < 0.001) \) and 72 h following the IIMD protocol \( (P = 0.004) \).

Compared to PRE, VAS-ACT was significantly elevated immediately post-IIMD \( (P < 0.001) \). Furthermore, when compared to PRE, VAS-ACT was significantly elevated at 24 \( (P < 0.001) \), and 48 h \( (P = 0.001) \). There was no significant difference in VAS-ACT at 72 h \( (P = 0.055) \). A
similar response was observed for VAS-PALP and VAS-PASS throughout the testing period (Figure 10).

Figure 10. Change in Visual Analogue Scale (VAS) for subjective soreness (0-100 mm) PRE, POST, 24, 48 and 72 h following the impact induced muscle damage (IIMD) protocol. VAS-PASS – VAS following a period of passive standing, VAS-ACT – VAS following a period of active bench step-ups, VAS-PALP – VAS following palpation of the impact sites. Data are mean ± SD. *Significant (*P <0.01) difference from PRE. Further, there were significant relationships observed between change in SJ-PP and soreness measures at 48 and 72 h (Table 3).
Table 3. Correlation between changes in squat jump peak power (SJ-PP) and soreness measured passively (VAS-PASS), following an active task (VAS-ACT), and on palpation of lower-limb musculature (VAS-PALP).

<table>
<thead>
<tr>
<th>SJ-PP</th>
<th>ΔPRE-POST</th>
<th>ΔPRE-24 h</th>
<th>ΔPRE-48 h</th>
<th>ΔPRE-72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS-PASS</td>
<td>-0.007</td>
<td>-0.228</td>
<td>0.535*</td>
<td>0.497*</td>
</tr>
<tr>
<td>VAS-ACT</td>
<td>-0.320</td>
<td>-0.256</td>
<td>0.524*</td>
<td>0.594*</td>
</tr>
<tr>
<td>VAS-PALP</td>
<td>0.220</td>
<td>-0.308</td>
<td>0.514*</td>
<td>0.528*</td>
</tr>
</tbody>
</table>

*level of significance $P < 0.05$.

3.6 DISCUSSION

The present study is the first to develop and implement a systematic method of producing IIMD in contact-sport populations, in the absence of EIMD. The implementation of this protocol resulted in IIMD and associated impairment in performance variables such as sprint speed and squat jump peak power, persisting for 48 h following the IIMD protocol. Similarly, the IIMD reduced perceived function and increased mood disturbance for a minimum of 48 h post-intervention. The IIMD intervention produced changes in subjective soreness that persisted for 72 h and which were related to change in performance at 48 and 72 h. Conversely, no significant differences in [hs-CRP] or [Mb] were observed when compared to baseline, primarily due to the highly individual response. Collectively, these data confirm that compared to EIMD, IIMD has independent adverse effects on a number of parameters including performance, soreness, mood and perceived function that can persist for upwards of 72 h.

The findings provide confirmation of the strong relationships previously observed between game-related collisions and performance impairment (18, 170). The change in squat jump peak power observed in the present study are substantially lower than those reported for
countermovement jump peak power immediately (9.6 vs. 31.2 %), and 24 h (8.1 vs. 23.3 %) following elite RL match-play (148). This is expected given that neuromuscular performance following match-play is likely to be negatively influenced by a number of factors including EIMD (46), as well as the physiological and cognitive/psychological aspects of fatigue (171) which were not relevant in the present study. Previous studies have reported altered neuromuscular function extending beyond 48 h of recovery following match-play in elite contact-sport populations (23). This is consistent with the present findings, suggesting this delayed athletic recovery from match-play may be, at least in part, due to the deleterious effects of IIMD. When exploring the limited research investigating IIMD in a controlled manner, decrements in jumping performance reported in the present study were slightly higher than those previously reported using similar methods immediately (~6 %) and 24 h (~5 %) following an IIMD protocol (21). One potential explanation is the difference in methods, with Johnston et al. (21) using primarily upper body collisions and wrestling in the context of a small sided conditioning game. Collisions resulted in a greater upper body performance decrement than lower body performance change immediately (~15 % vs. ~6 %) and 24 h post-exercise (~9 % vs. ~5%). These results suggest that the lower body would have experienced primarily EIMD through the running activity whilst the upper body would have been exposed to IIMD and EIMD through collisions and wrestling respectively. Compared to the upper body performance changes, peak power in the present study is lower immediately, and at 24 h following IIMD.

Changes in performance following collisions have been proposed to result from alterations in excitation-contraction coupling subsequent to the rise in intracellular oedema in animal models of IIMD (122). However, no significant change in ultrasound echo intensity, which is a marker of intramuscular oedema (158), nor in overall main effect for time and [hs-CRP], a systemic marker of inflammation were observed in the present study. Contributing to this lack of result is the high degree of interindividual variability and noise, both biological and artefact in the testing of GSA and [hs-CRP], as evidenced by the large standard deviations at each time point. Given each participant received the same intervention, this may also suggest a variable response based to IIMD on their individual physiology. These findings are thus
unclear and therefore further research is necessary to establish if this a contributing mechanism by which performance is influenced in contact-sport populations.

Interestingly, the change in 5 m sprint time was compromised to a significantly greater extent than that at 10 and 15 m immediately, and 24 h following the IIMD protocol. This suggests that initial acceleration mechanics and force generation capacity from a stationary start were influenced to a greater extent than the transition to top speed, at least in the initial 24 h following IIMD. This may, in part be due to the effects of IIMD being more pronounced at greater muscular forces, as has been identified in animal models (122). Consequently, muscle actions such as isometric and eccentric contractions that require greater muscular forces are influenced to a greater extent than repeated concentric or cyclic contractions. This may at least partially explain the divergent degree of performance decrement in the initial 5 m sprint, with greater horizontal forces required following the initiation of a sprint and decreasing force as velocity increases (172). Similarly, as velocity increases, so does the reliance on the elastic properties of the neuromuscular system and activation of the stretch-shortening cycle. Indeed, a similar magnitude of change was observed for SJ-PP and 5 m sprint time immediately following the IIMD protocol, suggesting that both measures, which require greater muscular forces and decreased activation of stretch-shortening cycle, were influenced to a comparable extent following IIMD. Currently, to the author’s knowledge, no research in human contact-sport populations has explored these effects following IIMD. As such, additional research is needed to confirm and further explore these data. Exploring other measures of neuromuscular performance, such as maximum voluntary contraction and muscle twitch characteristics, may further clarify the extent to which contractile function is influenced by IIMD (173). As such, additional research is needed to further explore this area of research, and identify factor(s) underpinning alterations in performance.

Whilst hs-CRP, a marker of inflammation, was elevated in response to IIMD, the overall effect was non-significant. The inflammatory response is initiated to facilitate tissue repair following muscle damage, with hs-CRP implicated in monocyte activation and the recruitment of leukocytes (174), key factors in promoting appropriate healing and recovery. Similar to the
findings on ultrasound echogenicity, the [hs-CRP] and [Mb] response to IIMD displayed high inter-individual variability. Given that each participant was prescribed the same IIMD intervention, one possible reason for the individual response may be a mismatch between the characteristics of the IIMD protocol and the participant’s ability to tolerate this ‘contact load’. This may have resulted in participants being exposed to contact loads that in some cases did not reach the upper limit of tolerance beyond which performance and biochemical parameters are noticeably decreased (120). For other participants, this threshold may have been exceeded and caused considerable changes. Similarly, individuals who have highly developed aerobic capacity and lower-body maximal strength capabilities recover indirect indices of muscle damage and performance faster when compared to with low aerobic capacity and maximal strength following RL match-play (175). These capacities may have had a protective effect on participants in the trial but given that these characteristics were not assessed, future research in IIMD may look to investigate the individual response to IIMD and stratify participants based on these variables.

The lack of an observed significant rise in Mb, a marker of muscle damage, in the present study is somewhat surprising, given that previous research has highlighted a strong relationship between [Mb] and game-related collisions (17). Indeed, the levels reported immediately following the present IIMD protocol (46.5 ± 20.4 ng·mL⁻¹) are far below those reported at 45 min following RU (~500 ng·mL⁻¹) (17) and 15 min following American Football match-play (~250 ng·mL⁻¹) (135). Similarly, concentrations are lower than those reported one hour following a simulated team-sport activity circuit (~170 ng·mL⁻¹) (125). A potential explanation for the lower concentrations observed in the present study include that the IIMD protocol was undertaken in the absence of EIMD, which would have likely contributed to amplified muscle damage and [Mb] in the aforementioned studies. Furthermore, [Mb] is influenced by intracellular release and circulatory uptake kinetics (135) and as such, the true peak concentrations may have occurred between the POST and 24 h testing time points and have been missed by the pattern of testing. Further research could look to implement testing more regularly in the hours following IIMD to accurately characterise the pattern and identify the peak in circulating muscle damage markers. Overall, the lack of significant findings with respect to [hs-CRP] and [Mb] further highlights the inter-individual variability that is inherent
in the primary and secondary muscle damage response, as often observed following EIMD (40).

Perceived soreness was elevated following the IIMD protocol and did not subside by the completion of the 72 h data collection period. Additionally, subjective soreness was lower following IIMD than data previously reported at the completion of elite RL match-play (170) and following a RL simulation protocol, involving no collisions (4). The pattern of the soreness response following IIMD is in contrast with the DOMS response which is often observed following EIMD (9). Typically following EIMD, soreness rises and peaks 24-48 h post-injury, before subsiding by 96 h (9). However, in the present study, soreness was observed immediately following the IIMD protocol, peaking 24 h later, and remaining elevated for at least 72 h. This finding is reflected in a recent study that observed soreness in the upper-, and lower-body were significantly related to total collisions in the initial days following RL match-play (19), with observed upper-, and lower-body soreness similar in recovery. This further highlights the divergence in the magnitude and progression of damage and associated symptomology between IIMD and EIMD (25, 28). An interesting finding was the relationship between soreness and squat jump performance at 48 and 72 h following IIMD. These data could suggest that soreness perceptions are highly related to the change in the ability to perform movement explosively in the days following IIMD. As such, these perceptions may provide an accurate monitoring tool to assess the change in performance following IIMD, especially in situations where testing performance may not be suitable or testing equipment limited. However, when soreness was at its peak immediately following and at 24 h following IIMD, there was no relationship with exercise performance. As such, the importance of this finding is unclear and more research is needed. These data may be of interest to coaches and athletes participating in these sports who regularly monitor perceptions of soreness and wellness (19).

Assessment of mood disturbance has been found to capture an individual’s mood at any given time point (22) and can be influenced by training stress and stress derived from competition (23). Disturbance scores in the present study were generally lower than those reported before
and after elite RU match-play (22, 23). This is likely due to the potential interactions between match performance, perceived stress, negative mood, and wellbeing (22), which were absent in the present study that did not incorporate a competition component. Previous research has shown that mood disturbance has an inverse relationship with changes in power output following match-play (22), however this was not observed in the present study. The present data suggest that IIMD has the potential to negatively affect mood. However, due to the transient nature in mood and the potential influence of lifestyle and perceptual factors on mood scores (22), factors outside the control of investigators that may influence mood must be acknowledged. Similarly, changes in perceived function were observed immediately following the IIMD protocol, with perceived function returning to baseline 48 h post-IIMD. However, these changes were modest and smaller than the nine point difference, previously determined as the smallest clinically important difference (167). As such the practical relevance of these findings remain unclear but the lack of a clinically significant change may be of interest to practitioners in determining the utility of these tests in identifying IIMD from subjective questionnaire methods.

The present study had a number of limitations which may have influenced the findings. Firstly, the force of impacts was not directly quantified. However, during pilot testing, the simulator was tested using a method for quantifying displacement from video (Dartfish Analyser, Dartfish, Switzerland) using a known reference in image (176) and subsequently velocity (velocity (m/s) = Δ displacement ÷ Δ time), acceleration (acceleration (m/s^2) = Δ velocity ÷ Δ time), and force (force (N) = mass x acceleration) data were derived using standard equations. The force involved per collision (~450 N) was substantially lower than that derived using published velocity and acceleration into contact data (~900 – 1100 N) from a similar recreational contact-sport athletic population (176). Given that this was a novel method of generating IIMD, safety of participants was paramount and as such increasing the system mass to produce greater impact forces equivalent to match-play was deemed unethical. Secondly, there was a lack of a control testing phase. However, to minimise the effects of initial testing on a subsequent testing bout, a substantial wash-out period, typically equal to or greater than six to eight months (75, 177) would have been necessary and this was not practical due to the availability of the athletes and limitations of the season structure. Finally,
participants were instructed to not undertake strenuous physical activity or engage in the use of recovery strategies prior to and for the duration of data collection. Although each participant maintained they had followed this directive, it is possible that they did not and therefore the potential that this may have influenced the findings of the present study cannot be discounted.

Future research may look to implement IIMD in contact-sport populations using the current method and explore existing protective equipment which may attenuate the magnitude and timeframe of damage responses. The use of prophylactic interventions like protective equipment, such as strapping, padding, and shin guards is commonplace in sports such as soccer, RU and Australian Rules Football (137, 140, 178), yet despite this widespread use, the efficacy of these strategies in preventing IIMD is yet to be firmly established. Longitudinal research has highlighted lower-limb padding as possibly reducing lower-limb haematomas in contact-sport athletes following match-play (142). The current method of inducing IIMD allows investigators to assess the effectiveness of these strategies in preventing the primary onset of IIMD, and therefore reduce the magnitude and duration of performance decrements, altered biochemical markers, and perceptual changes. Likewise, the current model of IIMD can be used by researchers and practitioners to explore recovery strategies which have already been examined in the context of promoting recovery from EIMD including hydrotherapy (54), cryotherapy (53), compression garments (55) and dietary interventions (26, 57, 59), amongst others. Given the current results and the potential differences between EIMD and IIMD (28), it is likely that certain recovery strategies could preferentially affect IIMD and exploration of novel methods should be an area of future research. Indeed, preliminary animal contusion research has highlighted polyphenols as one strategy with potential to reduce damage through attenuation of the inflammatory response (144), which was indirectly observed by elevated markers of inflammation 24 h following the IIMD protocol in the present study. Identification of effective strategies, both existing and novel, would be of interest to athletes and practitioners to promote the effective prescription of strategies to individuals who may be habitually exposed to a greater collision load. Another potential avenue worth exploring further in this field is if a dose-response exists between IIMD and dependent variables, that is after a certain frequency of collisions, does performance continue to
decrease or plateau. Future research is also necessary concerning exploration of the current model to investigate the ‘contact adaptation’ response (19, 134), which would be analogous to the repeated-bout effect in EIMD (179), possibly enhancing adaptation from an initial bout of IIMD on subsequent bouts.

In summary, the present investigation has demonstrated performance impairment that was sustained for at least 48 h following a standardised IIMD protocol. Subjective soreness, mood disturbance and perceived function followed a similar time course. Finally, biochemical markers of muscle damage and inflammation were not significantly changed throughout testing. Compared to EIMD, where soreness develops following a latency period, the time course of changes in subjective soreness displayed a divergent pattern with soreness being evident immediately following the IIMD protocol. Conversely, functional capacity was impaired immediately following IIMD, which is consistent with the time course of changes following EIMD. These data demonstrate that the IIMD protocol described here was safe for the athletes exposed to the intervention, who are habitually exposed to blunt force trauma, with no adverse injurious outcomes for the participants. The creation of a repeatable, systematic method of generating IIMD in contact-sport populations allows for the exploration of IIMD and its effects. The potential existence and time frame of this effect has been discussed in the literature but agreement remains equivocal (19). It would be of interest to athletes and practitioners as it may affect the periodisation of collision training in the macrocycle.
Chapter 4. Conclusions

From the available literature, it appears that contact-sport athletes are regularly exposed to situations which result in both EIMD and IIMD. Based on the purported differences in aetiology and progression there may be a divergent time course of implications on functional capacity, with the time course following IIMD presently unclear. To the author’s knowledge, this is the first study to examine the effects of IIMD on parameters including performance, biomarkers of muscle damage and inflammation, ultrasound-derived inflammation, soreness, mood and perceived function in the absence of exercise.

The present study found IIMD to have direct, negative implications on performance, soreness and mood that persisted through the days following the IIMD protocol. Changes in markers of muscle damage and inflammation were observed, however the highly variable response ensured that main effects for time were not significant. Sprint and squat jump performance were significantly attenuated in the 48 h following the IIMD protocol. Sprint performance was significantly slower at 5 m than at 10 and 15 m. The pattern of changes following IIMD contrasts somewhat with those observed following EIMD with performance changes exhibiting a similar time course whilst soreness displayed a divergent temporal response to that of EIMD. Potential reasons for this divergence have been discussed herein. This information is important in that it characterises the time course of changes related to IIMD which may be one component of post-match fatigue for contact-sport athletes. For athletes and practitioners, understanding that IIMD has the potential to negatively affect performance in the subsequent days following exposure to blunt-force trauma may assist in identifying interventions targeted at assisting recovery on an individual basis.

This research is novel and significant in that it describes and implements for the first time in human populations a unique approach to generating IIMD common experienced by rugby athletes and establishes a timeline of changes in a contact-sport cohort habitually exposed to
blunt force trauma. Establishment of a systematic method of delivering IIMD such as the one described and implemented in the present study allows researchers to explore interventions which may be used prophylactically to mitigate IIMD or assist in the recovery from IIMD. Similarly, this method can be used to explore and identify if a potential adaptive effect occurs from one bout of IIMD to a subsequent bout. Future research can use the method described here to examine and explore the various aspects of IIMD in order to enhance our understanding of muscle damage and recovery.
References


Appendix 1

Raw grouped values for squat jump peak power (SJ_PP) and five, ten, and fifteen meter sprint performance (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>POST</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SJ_PP (W)</strong></td>
<td>4183 ± 873.35</td>
<td>3785 ± 974.72</td>
<td>3861 ± 974.72</td>
<td>4050 ± 873.48</td>
<td>4189 ± 891.10</td>
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<tr>
<td><strong>FIVEm (s)</strong></td>
<td>1.08 ± 0.04</td>
<td>1.16 ± 0.04</td>
<td>1.14 ± 0.03</td>
<td>1.11 ± 0.05</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td><strong>TENm (s)</strong></td>
<td>1.84 ± 0.07</td>
<td>1.92 ± 0.05</td>
<td>1.92 ± 0.04</td>
<td>1.87 ± 0.06</td>
<td>1.86 ± 0.06</td>
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<tr>
<td><strong>FIFTEENm (s)</strong></td>
<td>2.52 ± 0.06</td>
<td>2.63 ± 0.07</td>
<td>2.60 ± 0.04</td>
<td>2.57 ± 0.06</td>
<td>2.54 ± 0.07</td>
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</tbody>
</table>