Nutritional Strategies and the Recovery of Muscle Function following Exercise Induced Muscle Damage: The Role of Complete Proteins in the form of Whey Protein Hydrolysate

Submitted by Kristoph Thompson to the University of Exeter as a thesis for the degree of Masters of Philosophy in Sports and Health Sciences, August 2014

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: …
Summary

The demands of training and competition can result in exercise-induced muscle damage (EIMD), and the rapid return to optimal performance is clearly advantageous in a range of sporting populations. EIMD is a well-documented phenomena with a variety of identified symptoms including muscle soreness, localised swelling, reductions in muscle strength and power, increased levels of creatine kinase, increased perceived exertion during activity and reductions in performance. A number of different strategies have been examined with the aim of limiting the occurrence of, and managing the symptoms of EIMD. Such strategies are currently employed by athletic populations, although the efficacy of the majority is based on equivocal or anecdotal evidence. Strategies include a range of therapeutic modalities, dietary interventions and activity regimens. The aim of this body of work was to investigate the efficacy of a particular dietary intervention; acute whey protein hydrolysate (WPH) ingestion, in ameliorating the effects of EIMD.

This thesis comprises eight chapters:

Chapter 1: Statement of problem and significance
Chapter 2: Aims and objectives
Chapter 3: Review of the literature
Chapter 4: Methodology and study design
Chapter 5: Study 1 - The effects of whey protein hydrolysate – a pilot study
Chapter 6: Study 2- The timing effects of whey protein hydrolysate
Chapter 7: Study 3 - The effects of whey protein hydrolysate and the repeated bout effect
Chapter 8: General discussion, conclusions and recommendations for further study

Chapter 3 outlines the findings of the relevant research into EIMD including the time course of the various symptoms associated with the phenomena, as well as the theorised mechanisms thought to give rise to the effects noted following bouts of strenuous activity. This chapter also includes a summary of the research into various modalities and strategies thought to either convey a protective effect against EIMD, or to ameliorate the effects of muscle-damaging exercise. While some the evidence for many strategies is equivocal (despite their widespread use amongst sporting populations), there are other strategies that show promise. Despite there being a body of evidence to support the use of practices to manage the effects of EIMD, the underlying mechanisms and beneficial effects are largely unclear. The general consensus among researchers being that more detailed investigation is required to elucidate the exact means by which strategies seem to have a beneficial effect on one or more symptoms of EIMD.

Chapter 4 summarises the methodology employed in all studies; namely that all studies would be undertaken in a double-blind, placebo controlled, repeated measures design. This chapter also outlines the various ethical considerations in inducing muscle damage, the ingestion of WPH, and the means of assessing the effects of EIMD on muscle soreness and performance. This chapter demonstrates that the protocols employed throughout all studies were in accordance with the ethical guidelines as set out by the University of Exeter at the time of data collection.

Chapter 5 consists of a pilot study undertaken to investigate the effects of one dietary intervention previously shown to offer protection against the effects of EIMD. The results of this pilot study indicate that the acute ingestion of WPH may serve to convey some protective effect against the performance decrements associated with EIMD (p<0.05). This finding is in line with previous research that report the beneficial effects of this dietary practice.
Chapter 6 examines the beneficial effects of WPH ingestion highlighted by the previous chapter, and to further examine whether there are any timing effects associated with acute WPH ingestion. This study seeks to address the limitations of the pilot study by employing a different strategy to induce muscle damage. In line with previous work, this study did continue to show some promise associated with the acute ingestion of WPH and a prevention of the decrements in muscle performance associated with EIMD within sprint performance between 5-10m (p<0.05). However, no timing effects were noted with acute WPH ingestion (pre vs. post a bout of eccentric exercise) (p>0.05).

Chapter 7 explores any possible interaction between acute WPH ingestion and the repeated bout effect (RBE) phenomena. It was hypothesised that the acute ingestion of WPH would serve to enhance the protective effect associated with the RBE as identified by previous research, leading to an enhanced recovery of muscle function. The results of this study highlighted the presence of a RBE, in line with previous work, as well as some beneficial effect in the recovery of muscle function (0-10m sprint performance; p<0.05), however no other benefits were shown in any other measures of performance or muscle soreness.

The final chapter of this thesis discusses the key findings of all three studies; namely that the acute ingestion of WPH may convey some protective effect in the recovery of muscle performance following EIMD (in particular sprint performance over 5-10m), and that there is some promise in the use of WPH in conjunction with the RBE (shown in the recovery of muscle function in 0-10m sprint performance). The use of WPH may be considered by some athletic populations in lessening the impact of EIMD. Recommendations for future research include examining the limitations of the studies detailed within this thesis: a larger statistical sample size; a more intense muscle-damaging exercise protocol; a more detailed examination of the physiological markers of muscle damage (i.e. CK); and more closely controlled dietary intake and physical activity throughout the study period (including the period between the initial and repeated bout of EIMD). Recommendations for wider research into
the area are the examination of continuous WPH supplementation vs. acute ingestion; and the exploration of a combination of strategies designed to reduce the effects of EIMD, such as WPH ingestion and the RBE.
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<tr>
<th>Abbreviation</th>
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<tr>
<td>AA</td>
<td>Amino Acids</td>
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<td>ANOVA</td>
<td>Analysis Of Variance</td>
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<tr>
<td>ADP</td>
<td>Adenosine Di-Phosphate</td>
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<tr>
<td>ATP</td>
<td>Adenosine Tri-Phosphate</td>
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<tr>
<td>BCAA</td>
<td>Branched Chain Amino Acids</td>
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<tr>
<td>Ca²⁺</td>
<td>Calcium Ions</td>
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<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<td>CK</td>
<td>Plasma Creatine Kinase</td>
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<td>CK-MM</td>
<td>Muscle-specific Creatine Kinase</td>
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<td>CM</td>
<td>Creatine Monohydrate</td>
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<td>CMJ</td>
<td>Countermovement Jump</td>
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<tr>
<td>COX</td>
<td>Cyclo-Oxygenase</td>
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<tr>
<td>CP</td>
<td>Creatine Phosphate</td>
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<td>CWI</td>
<td>Cold Water Immersions</td>
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<td>CWT</td>
<td>Contrast Water Therapy</td>
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<td>DOMS</td>
<td>Delayed Onset of Muscle Soreness</td>
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<td>EC</td>
<td>Excitation-Contraction</td>
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<td>ECC</td>
<td>Eccentric</td>
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<td>EE</td>
<td>Eccentric Exercise</td>
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<td>EIMD</td>
<td>Exercise-Induced Muscle Damage</td>
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<td>EMG</td>
<td>Electromyographic</td>
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<td>GCT</td>
<td>Ground Contact Time</td>
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<td>h</td>
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<tr>
<td>H⁺</td>
<td>Hydrogen Ions</td>
</tr>
<tr>
<td>HBO</td>
<td>Hyperbaric Oxygen Therapy</td>
</tr>
<tr>
<td>HMB</td>
<td>Hydroxy Beta-MethlyButyrate</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl-coenzyme A</td>
</tr>
<tr>
<td>HVPC</td>
<td>High Voltage Pulsed Current Electrical Stimulation</td>
</tr>
<tr>
<td>HWI</td>
<td>Hot Water Immersion</td>
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<tr>
<td>IL</td>
<td>Interleukins</td>
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<tr>
<td>KIC</td>
<td>Ketoisocaproic Acid</td>
</tr>
<tr>
<td>L-T</td>
<td>Length-Tension</td>
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LDH  Lactate Dehydrogenase
NSAID's  Non-Steroidal, Anti-Inflammatory Drugs
MENS  Microcurrent Electrical Neuromuscular Stimulation
MT  Musculo-Tendinous
MVC  Maximal Voluntary Contraction
NAC  N-acetylcysteine
NCV  Nerve Conduction Velocity
PGE2  Prostaglandin E2
PIT  Peak Isometric Torque
PNF  Proprioceptive Neuromuscular Facilitation
PPO  Peak Power Output
PRO  Protein
RER  Respiratory Exchange Ratio
RBE  Repeated Bout Effect
ROM  Range Of Motion
ROS  Reactive Oxygen Species
RPE  Rating of Perceived Exertion
SD  Standard Deviation
SEM  Standard Error of the Mean
SJ  Squat Jump
SOD  Superoxide Dismutase
SSC  Stretch Shortening Cycle
TENS  Transcutaneous Electrical Nerve Stimulation
TNFα  Tumour Necrosis Factor α
TAC  Total Antioxidative Capacity
uA  Microampere
VAS  Visual Analogue Scale
VT  Vibration Training
WPH  Whey Protein Hydrolysate
1RM  1 Repetition Maximum
1. Statement of problem and significance

1.1 Statement of Problem

Exercise-induced muscle damage is a well-documented phenomenon following exercise involving eccentric muscle actions (Friden et al., 1983; Newham et al., 1987).

Symptoms associated with EIMD include soreness, tenderness, changes in range of motion, strength loss, and release of muscle proteins such as creatine kinase (Eston et al., 1995). Soreness becomes apparent between eight and twenty four hours following muscle-damaging exercise (Smith, 1991; Newham, 1988; Hough, 1902). Sensations increase in intensity, reaching a peak between 24 and 72 hours post-EIMD (Bobbert, Hollander, and Huijing, 1986; Armstrong, 1984; Assmussen, 1956).

Strength losses in the muscles directly involved in the muscle-damaging bout are evident immediately after exercise. Research shown that the return to pre-exercise levels may occur over a period of hours (Newham et al., 1983; Davies and White, 1981), days (Byrne and Eston, 2002a; Hortobagyi et al., 1998; Golden and Dudley, 1992), or weeks (Sayers and Clarkson, 2001; Howell et al., 1993). The exact mechanisms that account for these differences is unclear, however differences in the magnitude of muscle damage, the protocol used to induce damage, or the population studied might in some part contribute.

EIMD is also associated with impaired muscle function and performance (Byrne and Eston, 2002a; Clarkson and Hubal, 2002; McHugh, 2003; Connolly, Eston and Gleim, 1999; Clarkson, Nosaka, and Braun, 1992; Brynes, Clarkson, White, Hsieh, Frykman, and Maughan, 1985; Armstrong, 1984). EIMD is far more likely to be experienced, and the associated functional impairments more severe, in individuals unaccustomed to regular physical activity than highly trained athletes (Lenn et al., 2002; Clarkson et al., 1992; Friden, Sfakianos, and Hargens, 1986), and among untrained than resistance-trained men (Newton et al. (2008). However EIMD does regularly occur among athletic populations, especially during periods of overreaching or overtraining (Gleeson, 1998; Kuipers, 1998).
Numerous studies have investigated a range of strategies and interventions (i.e. dietary interventions, pharmaceutical treatments, and therapeutic modalities) to prevent the signs and symptoms of EIMD from developing, or to alleviate them once present. A number of interventions have been shown to reduce the symptoms of EIMD and have been the subject of many reviews (Armstrong, 1984; Byrd, 1991; Howatson & van Someren, 2008). Strategies such as the use of nutritional supplementation (Buckley et al., 2010; Bryer and Goldfarb, 2006) compression sleeves (Kraemer et al., 2001), light exercise (Sayers et al., 2000; Gleeson et al., 2003), use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (Cannavino et al., 2003; Tokmakidis et al., 2003), massage (Farr et al., 2002), interferential therapy (Minder et al., 2002), hyperbaric oxygen therapy (Harrison et al., 2001), and cryotherapy (Paddon-Jones & Quigley, 1997).

These studies demonstrate limited effectiveness and as such, a sound and consistent treatment for EIMD has not yet been established. There are, therefore, no guidelines as to the optimum intervention, or the most appropriate dose/frequency to prevent/treat EIMD.

Despite the fact that EIMD is a commonly experienced and well-documented phenomenon, that can occur in any individual independent of fitness level, the exact mechanism(s) responsible is not completely understood. It is believed that the damage resulting from eccentric exercise is initiated by mechanical forces (Proske & Morgan, 2001) that disrupt the contractile component of the muscle at the level of the Z-line (Friden and Lieber, 1992). Other factors, such as calcium influx and reactive oxygen species, may initiate or contribute to the damage process (Kendall and Eston, 2002a; Gissel and Clausen, 2001). More recently, Tee et al. (2007) have proposed a model for the initiation of EIMD. This model states that damage initiation may be either metabolic or mechanical, or a combination of both, depending on the mode, intensity and duration of exercise and the training status of the individual. To date, no research has been able to conclusively attribute any one mechanism as the primary cause of EIMD or its related sensations; however, much of the research has documented events similar to those associated with an acute inflammatory response.

The benefits of minimising the subsequent decrements in performance associated with EIMD are of relevance to athletic populations. These
populations may presently use nutritional strategies to facilitate recovery of muscle function, and these could include protein ingestion. Although protein has been shown to facilitate muscle protein synthesis (in combination with resistance exercise) (Borsheim et al., 2002; Miller et al., 2003; Tipton et al., 2003), its effectiveness in attenuating EIMD is presently unknown.

1.2 Significance

The decrements in muscular performance associated with EIMD impact on the ability to perform sporting activities, as well as everyday tasks. Any intervention that was shown to minimise the effects of EIMD, and subsequently increase performance following muscle-damaging exercise, would be of great interest.

The effects of EIMD on strength and power result from alterations in the torque angular velocity relationship, having implications for those activities that emphasise high power outputs e.g. sprinting (Byrne and Eston, 2002). Additionally, the occurrence of reflex inhibition as a protective mechanism may hinder technique in common training modalities e.g. Olympic weightlifting; impairing training (Byrne et al., 2004). EIMD has further implications as sports involving high intensity intermittent activity, such as football and rugby, can result in muscle damage and the functional alteration outlined above (Thompson et al., 1999). As a result, research into EIMD serves to benefit a wide audience. Information elicited from these studies provides active individuals with valuable information concerning the benefits of whey protein hydrolysate ingestion with the goal of managing and treating the clinical signs and pain associated with EIMD.
2 Aims and Objectives

The overall aim of this body of work is to ascertain whether the acute administration of whey protein hydrolysate has any effect on the recovery of muscle function following a bout of exercise designed to elicit EIMD, further to previous research that has shown some efficacy of whey protein hydrolysate (Buckley et al., 2010; Tipton et al., 2004) in ameliorating the effects of EIMD.

Previous work has demonstrated the efficacy of branch chain amino acids (Howatson et al., 2012) and whey protein hydrolysate (Buckley et al., 2010; Tipton et al., 2004) in ameliorating the effects of EIMD. However, the impact of whey protein hydrolysate on the recovery of muscle function, the optimal timing of ingestion, and any interaction with the repeated bout effect, are at present unknown.

This body of work sets out to test the hypothesis that the ingestion of whey protein hydrolysate will lead to enhanced recovery of muscle function. The first objective is to determine the effects of acute WPH ingestion on the recovery of muscle function following EIMD. The second objective is to examine whether there are any timing effects associated with the acute ingestion of WPH in relation to the recovery of muscle function following EIMD. The third objective is to explore the effects of WPH ingestion with respect to the RBE phenomena to determine whether WPH enhances or blunts this response.
3 Review of Literature

The earliest known study of exercise-induced-muscle damage was published in 1902, in which Hough (1902) hypothesised the aetiology as being the result of micro-tears in the muscle. The phenomenon of EIMD continues to be the focus of much research and discussion, although the exact cause and optimal treatment have yet to be established.

Numerous studies have examined the various signs/symptoms of EIMD, as well as a range of interventions purported to prevent or minimise the effects of muscle-damaging exercise. A detailed discussion of each of these is beyond the scope of this work, therefore an account of those signs/symptoms and interventions (protein ingestion) relevant to the present body of work is provided. In addition, a brief summary of those interventions that may exert some influence is given as participants will be directed to refrain from partaking in these for the duration of the study period.

3.1 Eccentric Exercise

Although EIMD is experienced following any unaccustomed exertion, the typical means by which it has been induced in scientific studies is via subjecting muscle to high-force repetitive eccentric contractions during which the activated muscle is forced to elongate while producing tension (Chleboun et al., 1995; Newham, 1988; Friden et al., 1986; Komi and Viitasalo, 1977; Assmussen, 1956; Abbott, Bigland and Ritchie, 1952). Eccentric exercise is defined as the force generated by muscle activation involving lengthening of a muscle (Garrett and Tidball, 1988).

During eccentric activity, the force developed in the muscle is approximately 1.5-1.9 times greater than that developed during isometric contractions (Lombardi and Piazzesi, 1990; Edman et al., 1978); however, the total number of attached cross bridges in a strongly bound state is only ~10% greater than that during an isometric contraction (MacIntyre et al., 1996; Faulkner, Brooks, and Opiteck, 1993). As a result, the force is distributed over a smaller cross-sectional area of muscle, producing greater tension per active motor unit, thus increasing the risk for mechanical damage.
3.2 Clinical Signs and Symptoms

Symptoms associated with EIMD include soreness, tenderness, changes in range of motion, strength loss, and elevated levels of plasma creatine kinase (Eston et al., 1995). While there is an associated reduction in muscle function associated with EIMD, however, there is no evidence that there is any long-term damage or permanent reduction of muscle function (Armstrong, 1984). The following is an account of the various symptoms associated with EIMD, their associated timeframes, as well as the (theorised) mechanisms behind each.

3.2.1 Pain

Pain serves to protect by signalling injury to the tissues. However, the pain associated with EIMD initiates between 8 and 24 hours post-exercise, with peak levels occurring at around 24-72 hours (Newham, 1988; Bobbert et al., 1986). Since the soreness appears some time after the activity, it presumably does not function to prevent overuse during the bout in which the injury occurs (Armstrong, 1984).

The sensation of pain in skeletal muscle is transmitted by myelinated group III and unmyelinated group IV afferent fibres (Armstrong, 1984). Due to the myelination of the axons, the myelinated group III fibres display faster neural transmission rates whereas the unmyelinated fibres exhibit a slower transmission rate. Whereas unmyelinated axon conduction velocities range from about 0.5 to 10 m/s, myelinated axons can conduct at velocities up to 150 m/s (Purves et al., 2001). Myelinated fibres are believed to transmit sharp pain, whereas the unmyelinated fibres transmit dull aching pain more commonly associated with muscle damage (Armstrong, 1984).

The exact mechanism responsible for the delay in pain is not fully understood. However, according to Smith (1991) the biochemical explanation is related to the delay in macrophage entry into the injured area. In response to eccentric-based exercise, macrophages are present in large numbers at 24 and 48 hours after tissue injury. It is suggested that the sensation of pain was related to the synthesis of PGE2 by the macrophage. PGE2 directly causes the sensation of pain by activating pain afferents, most likely myelinated type III and unmyelinated type IV pain afferents (Ebbeling and Clarkson, 1989).
subsequent review by Malm and Proske (2001) explained that the current view of the mechanism responsible for the pain experienced post-exercise is as a result of nociceptor sensitisation (as a result of the tissue breakdown products) so that they respond to stimuli that would otherwise be non-noxious.

In his review, Malm (2001) also lends support to the potential for nociceptors in the pain mechanism, citing the work of Marchettini et al. (1996). However, Malm (2001) also proposes that pain may not be experienced as a result of muscle inflammation or swelling of the muscles fibres that may or may not be caused by muscle inflammation, as previously thought. He suggests that muscle pain could be inflicted by the release of substances from muscle cells, including PGE2, citing previous work by Stebbins et al. (1990), Dray and Perkins (1993) and Babenko et al. (1999). This lends support to the biochemical theory outlined previously. Malm also suggests a second possible explanation could be muscle swelling caused by other factors such as increased protein metabolism in the muscle cells and the subsequent increase in osmotic pressure. Interestingly, Malm (2001) suggests the presence of pain serves to allow adequate time for muscle recovery and adaptation by decreasing the likelihood of undertaking strenuous activity.

3.2.2 Vertical Jump

The recovery process of the vertical jump appears to differ somewhat to that of measures of power. Avela et al. (1999) and Horita et al. (1999) reported in immediate decline in performance followed by small recovery, before further reductions in performance noted 48h-72h post-exercise. The secondary decline in performance was suggested by the authors as being associated with the secondary, inflammatory phase, of EIMD. When EIMD was induced using plyometric exercise, reduction in maximal force production, ground reaction forces, stretch-reflex sensitivity, muscle stiffness, and drop jump performance were impaired (Avela et al., 1999; Horita et al., 1999). Byrne and Eston (2002b) showed that the period of reduced performance differed according to the type of jump; squat jump performance was affected to a greater extent than countermovement or drop jump, suggesting that the stretch shortening cycle possibly attenuates the decline in performance associated with EIMD (Byrne et al., 2004).
3.2.3 Sprint Performance

There has been limited study into the effects of EIMD on sprinting performance; the first by Semark et al. (1999) reported no evidence to suggest that muscle damage was detrimental to sprint performance at 5, 10, 20, and 30m from a standing start, measured at 12h, 24h, 48, and 72h following muscle-damaging exercise amongst trained subjects. Pain was significantly elevated at 24h and 48h however no significant change in CK was noted over the study period. Later work by Twist and Eston (2005) employed a plyometric exercise protocol to induce EIMD, resulting in significant increases in muscle soreness and CK activity. Performance in repeated sprints (10 x 10 m sprints from a standing start) was significantly reduced at 30 min, 24 h and 48 h compared to baseline, returning to baseline values by 72h. Sprint times over 10 m were higher (P<0.05) at 30 min, 24 h and 48 h compared to baseline (1.96s) with values corresponding to 2.01s, 2.02s and 2.01s at 30 min, 24 h and 48 h, respectively. These findings are in support of later work by Highton et al. (2009) who reported a significant increase in sprint time (6% over 5m and 5% over 10m) and agility time (8% increase in 505-agility test time). The concurrent nature of the findings of the latter studies, combined with the lack of an increase in CK, would suggest that the exercise protocol used in the preliminary research was not sufficient to induce damage, and that EIMD significantly impacts sprint performance for up to 3 days following exercise.

3.2.4 Evaluating the magnitude of muscle damage

Due to the differences in time frame between the onset and dissipation of soreness, and functional impairments, DOMS should not be used as an indicator of the magnitude and functional impairments (Rodenburg et al., 1993; Jones et al., 1986). Warren et al., (1999) suggest that muscle function provides the most effective means of assessing the effects of eccentric exercise, particularly when considering damage in the context of athletic performance.
Twist and Eston (2005) propose that the effects of eccentric exercise on performance measures, such as those employed within sport, are of particular interest when attempting to apply and findings to an athletic population.

3.3 Theorised Mechanisms

3.3.1 Metabolic Waste Accumulation

Eccentric exercise is thought to cause either ischemia or hypoxia, leading to changes in ion concentration, metabolic waste accumulation and adenosine triphosphate deficiency, which ultimately result in damage similar to that seen in EIMD (Byrnes & Clarkson, 1986; Gordon & Ridgeway, 1978; de Vries, 1966).

This theory was initially based on the assumption that lactic acid continues to be produced following exercise. Assmussen (1956) stated that this was due to an excessive build-up of lactic acid in the muscle following strenuous eccentric activity. This theory has been largely rejected as the higher degree of anaerobic metabolism and lactic acid production associated with concentric muscle contractions has failed to result in the sensations similar to those experienced by individuals following eccentric muscle contractions (Newham et al., 1983). Eccentric exercise, which has been shown to produce the largest amount of muscle soreness in individuals, requires relatively low energy expenditure. The energy used (per unit area of active muscle) is less in eccentric exercise than concentric exercise (Newham et al., 1983). Thus, if metabolic waste accumulation were the cause of EIMD, symptoms would be expected to be greater after exercise with a higher anaerobic metabolic cost (concentric exercise) (Szymanski, 2001), which has been shown not to be the case (Newham et al., 1983). Comparisons of uphill and downhill running also serves to illustrate this notion, with uphill running showing a higher metabolic cost and little signs of damage when compared with downhill running which resulted in damage at a lower metabolic cost (Armstrong et al., 1983). The fact that peak soreness is experienced 24-72 hours after eccentric exercise (Newham, 1988; Bobbert et al., 1986) serves as further evidence against this theory. At this time blood lactate concentration has already returned to pre-exercise levels.
(Schwane et al., 1983). For these reasons, it is unlikely that lactic acid accumulation is the underlying mechanism causing EIMD.

3.3.2 Mechanical Stress

Eccentric contractions are capable of generating more force than isometric and concentric contractions, requiring less energy per unit of torque (Abbott, Bigland and Ritchie, 1952; Bigland-Richie, Woods, 1976). Lengthening of sarcomeres is non-uniform during eccentric contractions which results in some myofilaments being stretched and unable to overlap within the sarcomere (Talbot and Morgan, 1996). When filaments are stretched beyond the point of overlap, passive structures are placed under greater stress, resulting in Z-band streaming (Lieber and Frieden, 1999). Excessive tension may cause failure of the structures, resulting in reduced ability to generate force. Evidence from electron microscope examinations show sarcomeres out of register with one another, Z-line streaming, regions of overextended sarcomeres or half-sarcomeres, regional disorganisation of the myofilaments and t-tubule damage (Morgan & Allen, 1999).

3.3.3 Intracellular Ca$^{2+}$

Eccentric exercise results in reduction of sarcoplasmic reticulum membrane integrity and the influx of Ca$^{2+}$ (Nielsen et al., 2005). The influx of Ca$^{2+}$ causes damage to the cytoskeleton, sarcoplasmic reticulum, mitochondria, and myofilaments (Gissel and Clausen, 2001; Byrd, 1992). Proteolytic and lipolytic pathways are activated, leading to the degradation of cell membrane and the leakage of intramuscular proteins into the blood (Warren, Lowe and Armstrong, 1999).

3.3.4 Free Radical Damage

Contracting skeletal muscles generate free radicals, and prolonged intense exercise can result in oxidative cellular damage (Reid et al., 1992; Alessio et al. 1988). It has been suggested that free radicals are associated with direct tissue damage but may also increase the inflammatory response, which in turn may result in further cellular damage (Best et al., 1999).
It is unlikely that free radicals alone are central to any theory of EIMD, particularly when eccentric contractions are employed to induce damage. Eccentric exercise results in significantly greater muscle damage than concentric exercise, yet requires less oxygen consumption. If free radicals were the primary cause of damage, signs and symptoms would be greater following those activities with a higher oxygen consumption (Lastayo et al., 1999).

3.3.5 Proposed Model of Damage
Armstrong (1990) has proposed an integrated model of muscle damage which defines four stages: (i) initial events; (ii) autogenic processes; (iii) phagocytic stage; (iv) regenerative stage. It should be noted that these stages overlap, and the exact mechanisms responsible are unknown (Kendall and Eston, 2002a). The initial events are thought to be a result of either mechanical or metabolic in nature (Pyne, 1994; Armstrong, 1990). Armstrong (1990) suggests that mechanical stress results in disruption to the sarcolemma (causing calcium entry), the sarcoplasmic reticulum (resulting in impaired calcium sequestration) and myofibrillar structures. Metabolic events include increased temperature, lowered pH, insufficient mitochondrial respiration and oxygen free radical production (Armstrong, 1990).

The rate of ATP splitting could be reduced and Ca^{2+} pumping by sarcoplasmic reticulum slowed due to a reduction in local ATP and/or reduction in the free energy from hydrolysis of ATP, due to increased ADP (Byrd, 1992). A decrease in pH affects the ability of the sarcoplasmic reticulum to take up Ca^{2+}, which has been attributed to H^+ and Ca^{2+} ions competing for the Ca^{2+} binding site on the ATPase pump (Byrd, 1992). Increased temperature (above 38°C) has been shown to uncouple the Ca^{2+} stimulated ATPase activity from Ca^{2+} transport by the sarcoplasmic reticulum (Byrd, 1992), and may also alter the fluidity of the lipid membrane surrounding the ATPase pump and alter its ability to sequester Ca^{2+} (Byrd, 1992). Tee et al. (2007) have recently proposed a model for the initiation of EIMD; however this has yet to substantiated. This model states that damage initiation may be either metabolic or mechanical, or a combination of both, depending on the mode, intensity and duration of exercise and the training status of the individual.
The autogenic processes that follow originate in the muscle fibres and although the underlying mechanisms are unknown the loss of intracellular Ca\(^{2+}\) homeostasis could play a primary role (Armstrong, 1990). Processes proposed to explain how muscle could be damaged following elevation of intramuscular calcium content include: stimulation of calcium-activated proteases, activation of lysosomal proteases, mitochondrial overload and activation of lipolytic enzymes (Kendall and Eston, 2002a). McArdle et al., (1992) consider the two most important processes to be the activation of lipolytic enzymes and calcium-activated proteases, calpain.

An increase in intracellular calcium causes damage to the myofilaments of skeletal muscle (Duncan, 1978). It has been proposed that elevated Ca\(^{2+}\) causes a release of muscle enzymes through activation of phospholipase A\(^2\), which in turn may result in injury to the sarcolemma via the production of leukotrienes and prostaglandins through the formation of ROS and/or the release of lysophospholipids that exert a detergent-like effect (Armstrong, 1990). This affects the integrity of the membrane resulting in a 'leaky' membrane, loss of intracellular enzymes and an efflux of lysosomal enzymes (Jenkins, 1988). Significant increases in calcium causes ultrastructural changes in the muscle cell, including swollen and disrupted mitochondria, dilated t-tubules and sarcoplasmic reticulum, general cellular oedema and disruption of the myofilaments (Byrd, 1992).

Elevated Ca\(^{2+}\) has also been associated with disruption in the excitation-contraction coupling process, which in turn has been related to the reduction in force associated with eccentric exercise (Ingalls et al., 1998). E-C coupling is the sequence of events that begins with the movement of the action potential along the plasmalemma and ends with the release of calcium from the sarcoplasmic reticulum (Ingalls et al., 1998). Animal experiments (Ingalls et al., 1998; Warren et al., 1993) have confirmed a reduced rate of calcium release from the sarcoplasmic reticulum and greater reductions in maximally activated tetanic force versus maximally activated caffeine-activated force, which indicates the loss of force after eccentric contractions is a result of a failure to fully activate the contractile machinery rather than damage. The failure of the
E-C coupling had profound effects in these studies; investigators (Ingalls et al., 1998; Warren et al., 1993) estimated that at least 75% of the reduction in maximal titanic force was due to E-C coupling failure immediately post-exercise and responsible for at least 57% of the reduction at 5 days post-exercise.

EIMD is associated with disturbances in Ca\(^{2+}\) homeostasis, prompting the activation of the non-lysosomal protease Calpain. Belcastro et al. (1998) have proposed a calpain hypothesis, suggesting that calpain, a non-lysosomal protease results in the selective proteolysis of various contractile, metabolic, and structural elements. They also believe that calpain, or the resultant peptide fragments, may be associated with the neutrophil chemotaxis reported to occur during or following exercise, aiding the inflammatory response and repair process.

Interleukins 1, 2, and 6 and tumour necrosis factor α (TNFα) are believed to be the principle mediators of inflammation (Imura et al., 1996). In particular IL-6, which has been shown to increase in concentration following exercise and muscle injury, is considered to influence inflammation as well as play a role in stimulating protease synthesis (Tidball, 1995).

Leucocytes (neutrophils and monocytes/macrophages) are thought to perform a number of functions during the inflammatory stage of muscle damage; within the muscle damage and repair cycle of these cells: (i) attack and break down debris (neutrophils and macrophages); (ii) removal of cellular debris (macrophages); and (iii) regeneration of cells (macrophages) (Clarkson and Sayers, 1999; Tidball, 1995). Neutrophils release a number of chemoattractants, amplifying the response by recruiting additional neutrophils and mononuclear cells, and generating ROS (Pyne, 1994). Macrophages produce ROS and give rise to cytokines, which in turn might exacerbate damage by potentiating cytotoxic mechanisms of other inflammatory cells to enhance free radical production and enzyme release (Evans and Cannon, 1991). Some macrophages may play a role in muscle repair; in animal studies ED1+ cells have been shown to act as phagocytes and ED2+ cells regulate the consequent repair process (Clarkson and Sayers, 1999).
During the phagocytic phase of muscle damage there is an associated division of surviving satellite cells, which mature into myoblasts and fuse to form new myotubes (Kendall and Eston, 2002a). It does appear that invasion by macrophages is integral to regeneration, particularly with respect to satellite cell division (Merly et al., 1999).

3.4 Interventions to Attenuate EIMD
A number of interventions have been proposed in order to minimise the occurrence of damage, with varying degrees of success. One such intervention that shows promise is the ingestion of dietary protein. The repeated bout effect is another strategy that has been shown to result in diminished EIMD. These two strategies form the basis of this body of work, therefore an account of the evidence to support their use is included below.

3.4.1 Protein
Eccentric exercise results in both protein degradation and protein synthesis (Sorichter et al., 1999). Therefore, the ingestion of dietary proteins may exert an influence by increasing protein synthesis following exercise, or reducing the extent of proteolysis (Rennie and Tipton, 2000). While the exact mechanism is unclear, a number of authors have shown beneficial effects on minimising the effects of EIMD and/or speeding the restoration of muscle function to pre-exercise levels.

3.4.2 Whey Protein Hydrolysate
Acute ingestion of protein after exercise results in muscle anabolism following resistance exercise (Tipton et al., 2004). There is also evidence which suggests that protein hydrolysates can accelerate the repair of damaged tissue (Lee et al. 2006). Buckley et al. (2010) hypothesised that since the effects of eccentric exercise are associated with tissue damage, protein hydrolysates may speed recovery of force generating capacity following muscle damaging exercise. Subjects ingested of 25g of whey protein isolate hydrolysate immediately after 100 maximal eccentric contractions of the knee extensors, again at 6h and 22h post-exercise. Compared to placebo and 25g of whey protein isolate, peak isometric torque (PIT) improved rapidly following
supplementation with whey protein hydrolysate. PIT decreased 23% following eccentric exercise, and remained suppressed in the placebo and whey protein isolate groups, but recovered fully in the whey protein isolate hydrolysate group by 6h post-exercise. The exact mechanism responsible is unknown; with hydrolysate having no effect on muscle soreness, CK, and plasma TNF α. Further study is needed into the mechanism by which hydrolysate was able to elicit an improvement in the recovery of muscle function in these investigations.

3.4.3 Repeated Bout Effect
A bout of eccentric exercise performed prior to further eccentric exercise has been shown to convey significant protective effects, referred to as the repeated bout effect (RBE). Early studies demonstrated the effects of the RBE on indirect indices of damage (CK, muscle soreness and function) (Mair et al. 1995; Newham et al. 1987; Byrnes et al. 1985).

The proposed mechanisms were the subject of a review by McHugh et al. (1999a). It was posited that the adaptation was a result of neural, connective tissue or cellular adaptations. However, other possible mechanisms include adaptation in excitation-contraction coupling or adaptation in the inflammatory response. The neural theory states that following the repeated bout an increase in motor unit activation and/or a shift to slow twitch fibre activation distributes the contractile stress over a larger number of fibres. According to the connective tissue theory the repeated bout effect results in remodelling of the intermediate filaments and/or increased connective tissue. The cellular theory states that there is an increase in the number of sarcomeres in series which reduces sarcomere strain, limiting damage.

McHugh et al. (1999a) concluded that it is unlikely that one theory can explain all of the various observations of the repeated bout effect found in the literature. The RBE occurs in electrically stimulated contractions in an animal model, precluding neural adaptation exclusively. Connective tissue and cellular adaptations are unlikely explanations when the repeated bout effect is demonstrated prior to full recovery, and when the fact that the initial bout does not have to cause appreciable damage in order to provide a protective effect is
considered. The authors stated that the repeated bout effect occurs through the interaction of various neural, connective tissue and cellular factors that are dependent on the particulars of the eccentric exercise bout and the specific muscle groups involved.

More recently, McHugh (2003) conducted a follow-up review outlining advances in the potential mechanisms behind the RBE. It was stated that although there is some evidence to suggest that the RBE is associated with a shift toward greater recruitment of slow twitch motor units, it is more likely that a peripheral, non-neural adaptation predominates since the RBE has been demonstrated with electrically stimulated contractions.

With respect to mechanical adaptations there is evidence that both dynamic and passive muscle stiffness increase with eccentric training but there are no studies on passive or dynamic stiffness adaptations to a single eccentric bout. It is suggested that the role of the cytoskeleton in regulating dynamic stiffness is a possible area for future research.

With respect to cellular adaptations there is evidence of longitudinal addition of sarcomeres and adaptations in the inflammatory response following an initial bout of eccentric exercise. Inflammatory adaptations are thought to limit the proliferation of damage that typically occurs in the days following eccentric exercise. McHugh (2003) concludes that a unified theory explaining the mechanism or mechanisms for this protective adaptation remains elusive.

The prophylactic effect of the RBE has been shown with relatively high-volume eccentric contractions. Brown and colleagues (1997) set to investigate whether a lesser initial bout might convey a similar protective effect. It was shown that 10 maximal eccentric contractions of the knee flexors resulted in no change in CK activity, whereas higher volume contractions did, indicating significantly less muscle damage. However a single bout of relatively few maximal eccentric contractions is sufficient to bring about an adaptation, and increasing the number of eccentric muscle repetitions did not result in an increased prophylactic effect on skeletal muscle. These findings are supported by those
of Clarkson and Tremblay (1998) and Nosaka et al. (2001a). The former showing that 24 maximal eccentric contractions of the elbow flexors attenuated damage from a subsequent bout of 70 contractions, even though minimal damage was induced. The prophylactic effect was evident with as little 2 or 6 maximal eccentric repetitions during an initial bout, as shown in the latter investigation, prior to a repeated bout of 24 contractions of the elbow flexors. This hypothesis was supported by a more recent investigation by Howatson, van Someren, and Hortobágyi (2007) that demonstrated no significant difference between the protective effect conveyed by a low volume (10 maximal eccentric contractions) as compared to a high volume (45 maximal eccentric contractions) initial bout protocol. They did note however, that the magnitude of the protective effect appears to lessen following the lower volume bout as compared with a bout of higher volume. These authors suggest that the protective effect is as a result of neural changes, independent of the volume of the initial exercise bout.

Nosaka et al. (2001a) sought to examine the length of time that the RBE might last. Subjects performed two bouts of eccentric exercise of the elbow flexors separated by either 6, 9, or 12 months. Recovery of maximal isometric force was faster following a second bout performed after 6 or 9 months, as well as reduced soreness and smaller increases in upper arm circumference, CK and T2 relaxation time. The authors concluded that the RBE lasts at least 6 months but is lost between 9 and 12 months.

It does appear that in order to convey a protective effect, the prior bout must be maximal in nature (yet not necessarily inducing considerable damage), as shown by Nosaka and Newton (2002).

The majority of early research allowed sufficient time for markers of damage to return to resting levels (approx. 2 weeks), however Nosaka and Newton (2002) examined the effects of subsequent bouts of eccentric exercise repeated 2 and 4 days after the initial bout. Repeated bouts did not result in further muscle damage or hinder recovery, findings which were mirrored by Chen (2003). Force production was significantly reduced in the subsequent bouts, suggesting
that the force produced was insufficient to cause further damage. If these findings were to be applied in a sporting context it is evident that subsequent training will not result in further damage, however if the goal is to elicit adaptation by overloading the musculature, the force generated is unlikely to be sufficient to result in positive adaptation. Further research into this possibility is suggested by Howatson and van Someren (2008) in their review of the literature.

In a recent study by Falvo et al. (2008), the RBE was shown to be absent among resistance trained men. Repeated eccentric bouts of the barbell bench press exercise (100 repetitions) were performed separated by 14 days. The authors concluded that a prior bout of eccentric exercise does not confer a RBE for indirect markers of muscle damage, however the effects of EIMD are most pronounced in novel, unaccustomed exercise and therefore unlikely to be evident in resistance trained men. Furthermore, as shown by Nosaka et al. (2001a), the prophylactic effects of the repeated bout can be conveyed for up to nine months and the magnitude is reduced each time, therefore the RBE is likely to have already occurred in resistance trained subjects in previous training. For these reasons, the absence of the RBE in resistance trained subjects is expected. The presence of a RBE may be better examined through the eccentric actions of a less commonly stressed muscle group, as the pectoralis major is likely to feature heavily in the majority of sporting and recreational resistance training programmes.

Newton et al. (2008) have shown that resistance trained men are appreciably less susceptible to EIMD than untrained men. It was noted that the resistances employed by those engaged in regular resistance training correspond to 80% or greater of their concentric 1 repetition maximum (1RM) yet this represents considerably less of their ability to generate force eccentrically, suggesting that the majority of eccentric work is performed at a sub-maximal level. Yet it would appear this training did convey a protective effect. To be classed as resistance trained, subjects were required have been engaged in resistance training for 1 year with a frequency of at least three sessions per week. These resistance trained subjects showed significantly smaller changes in maximal voluntary
isometric and isokinetic torque, range of motion, upper arm circumference, plasma creatine kinase activity; and faster recovery of muscle function compared to the untrained group.

A recent addition to the literature by Chen et al. (2009) sought to examine the effects of multiple repeated bouts since little is known about the RBE of more than two bouts of eccentric contractions. In this study 30 maximal eccentric contractions of the elbow flexors were performed every four weeks for four bouts. The authors showed that the first bout confers the greatest adaptation but further adaptation is induced when the exercise is repeated more than three times. The results show that recovery was significantly faster after the second to fourth bouts compared with the first bout, but a significant difference amongst the second or third bout and fourth bout was evident only for isometric and concentric strength and ROM. The authors concluded that additional protective adaptation was conferred by performing the eccentric exercise more than three times, as the magnitude of decrease in muscle strength and ROM immediately after exercise was significantly smaller for the fourth bout compared with the others.

The prophylactic effect of the repeated bout has been consistently shown in a number of studies, perhaps showing greater efficacy than any other intervention. Low-volume, high-intensity eccentric contractions appear sufficient to convey a protective effect, however should this be applied in a sporting context, sufficient time should be allowed for recovery of muscle function as impaired force production may prohibit positive training adaptations.

3.4.4 Additional Interventions
A range of additional interventions have been studied to assess their effectiveness in either lessen the initial damage following EIMD or to speed the restoration of muscle function to baseline levels. These are summarised in the table below.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Subjects</th>
<th>Study design</th>
<th>Treatment</th>
<th>Measures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryer and Goldfarb (1999)</td>
<td>N=18,</td>
<td>Randomised, placebo controlled</td>
<td>3g/day Vitamin C for 2wks prior to and 4 days following EIMD</td>
<td>Muscle strength, range of motion, CK, gluthathione ratio, muscle soreness</td>
<td>Treatment group experienced significantly lower muscle soreness (P = 0.023) post exercise (immediately-post, 4hrs and 24hrs) and gluthathione ratio at 4hrs and 24hrs.</td>
</tr>
<tr>
<td>Silva et al. (2009)</td>
<td>N=29</td>
<td>Randomised, single-blind, placebo controlled</td>
<td>10mg/kg/day for 2wks prior to and 7 days following EIMD</td>
<td>Muscle soreness (MS), lipoperoxidation, protein carbonylation, TNFα, IL-10</td>
<td>Some significant effects on IL-10 on day 7 however no significant effects noted for any other measures.</td>
</tr>
<tr>
<td>Mastaloudis et al. (2006)</td>
<td>N=22</td>
<td>Randomised, placebo controlled</td>
<td>1000mg/day Vitamin C and 300mg/day of Vitamin E supplemented</td>
<td>Plasma alpha-tocopherol, ascorbic acid, creatine kinase, lactate dehydrogenase,</td>
<td>No significant treatment effect noted.</td>
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</tbody>
</table>
for 6 weeks prior to EIMD
maximal voluntary contraction of the hamstring and quadriceps

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Design</th>
<th>Intervention (Duration)</th>
<th>Outcome Measures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jakeman and Maxwell (1993)</td>
<td>24</td>
<td>Randomised, placebo controlled, double blind</td>
<td>400mg/day Vitamin C or Vitamin E for 21 days prior to and 7 days following EIMD</td>
<td>MVC and ratio of force generated at 20Hz &amp; 50Hz tetanic stimulation.</td>
<td>Vitamin C shown to have some (P&lt;0.05) beneficial effect on recovery of MVC at 24hrs.</td>
</tr>
<tr>
<td>Su et al., (2008)</td>
<td>16</td>
<td>Randomised, placebo controlled, double blind</td>
<td>80mg allicin for 14 days prior to and 2 days following EIMD</td>
<td>CK, LDH, IL-6, SOD, perceived muscle soreness</td>
<td>Treatment group experienced significantly lower plasma levels of CK, IL-6, and reduced perceived muscle soreness after exercise (P&lt;0.01)</td>
</tr>
<tr>
<td>Saunders et al. (2007)</td>
<td>13</td>
<td>Counterbalanced, randomized, double blind</td>
<td>Carbohydrate and protein in combination vs. carbohydrate-only</td>
<td>CK</td>
<td>CK did not significantly increase immediately post-exercise in the CHO-PRO condition, whereas it did in the CHO condition.</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Design</td>
<td>Interventions</td>
<td>Outcomes</td>
<td>Findings</td>
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<tr>
<td>Betts et al. (2009)</td>
<td>17</td>
<td>Randomised, crossover</td>
<td>Carbohydrate vs carbohydrate and protein consumed for 4 hrs following EIMD</td>
<td>Peak Isometric Torque, CK</td>
<td>No significant difference shown between groups.</td>
</tr>
<tr>
<td>White et al. (2008)</td>
<td>27</td>
<td>Randomised, placebo</td>
<td>Carbohydrate and protein consumed either before or after EIMD</td>
<td>CK, MVC and muscle soreness</td>
<td>No significant difference noted</td>
</tr>
<tr>
<td>Paddon-Jones et al.</td>
<td>17</td>
<td>Randomised, placebo</td>
<td>40mg/kg/d of HMB for 6 days prior to and 10 days following EIMD</td>
<td>Muscle soreness, upper arm girth, muscle torque</td>
<td>No significant difference noted</td>
</tr>
<tr>
<td>van Someren et al.</td>
<td>6</td>
<td>Randomised, crossover,</td>
<td>HMB (3d/day) and KIC (0.3g/day) for 14 days prior to EIMD</td>
<td>1RM, CK, soreness, ROM</td>
<td>Supplementation shown to have some significant effect on markers of muscle damage (P&lt;0.05)</td>
</tr>
<tr>
<td>Wilson et al. (2009)</td>
<td>16</td>
<td>Randomised, crossover,</td>
<td>3g HMB pre or post EIMD</td>
<td>CK, muscle soreness, LDH, MVC</td>
<td>No significant effect noted</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Designation</td>
<td>Intervention</td>
<td>Outcome Measures</td>
<td>Results</td>
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<tr>
<td>Rawson et al. (2001)</td>
<td>23</td>
<td>Randomised, placebo controlled, double blind</td>
<td>20g/day creatine for five days prior to EIMD</td>
<td>MVC, ROM, circumference, muscle soreness, muscle serum proteins</td>
<td>No significant effect noted</td>
</tr>
<tr>
<td>Rawson et al. (2007)</td>
<td>22</td>
<td>Randomised, placebo controlled</td>
<td>0.3g/kg/day for five days pre and 0.03g/kg/day for five days post EIMD</td>
<td>MVC, ROM, soreness, C-reactive protein, CK, lactate dehydrogenase</td>
<td>No significant effect noted</td>
</tr>
<tr>
<td>Santos et al. (2004)</td>
<td>34</td>
<td>Randomised, placebo controlled, double blind</td>
<td>5g/day creatine for five days prior to EIMD</td>
<td>CK, LDH, TNFα, PGE2</td>
<td>Significant reductions in CK, TNF-α, PGE2 in treatment group (P&lt;0.05)</td>
</tr>
<tr>
<td>Rosene et al. (2009)</td>
<td>20</td>
<td>Randomized, placebo controlled</td>
<td>20g/day for seven day followed by 6g/day for 23 days</td>
<td>CK, LDH, MVC, ROM, muscle soreness</td>
<td>Significant (p&lt;0.05) treatment effect for isometric force</td>
</tr>
<tr>
<td>Hasson et al. (1993)</td>
<td>20</td>
<td>Randomised, placebo controlled</td>
<td>400mg Ibuprofen 4h before or immediately after EIMD</td>
<td>Muscle soreness, CK, knee extensor torque</td>
<td>Treatments group displayed significantly (p&lt;0.05) reduced pain and strength loss as compared to placebo at 24h.</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Design Type</td>
<td>Interventions</td>
<td>Outcomes</td>
<td>Results</td>
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<tr>
<td>Sayers et al. (2001)</td>
<td>48</td>
<td>Randomised, placebo controlled</td>
<td>25mg or 100mg prior to EIMD</td>
<td>Muscle soreness, maximal isometric force, myoelectric activity</td>
<td>Treatment conveyed significant effect on muscle soreness and force recovery (p&lt;0.05)</td>
</tr>
<tr>
<td>O'Grady et al. (2000)</td>
<td>54</td>
<td>Randomised, double blind, placebo controlled</td>
<td>150mg diclofenac sodium twice daily for 27 days</td>
<td>CK</td>
<td>Treatment group demonstrated significantly lower CK scores (p&lt;0.03)</td>
</tr>
<tr>
<td>Tokmakidis et al. (2003)</td>
<td>19</td>
<td>Randomised, double blind, placebo controlled</td>
<td>400mg ibuprofen every 8hrs for 48hrs following EIMD</td>
<td>Muscle soreness, CK, WBC, MVC, vertical jump</td>
<td>Treatment group experienced significantly lower muscle soreness and CK (p&lt;0.05), however now significant effect on any other measures</td>
</tr>
<tr>
<td>Jayaraman et al. (2004)</td>
<td>32</td>
<td>Randomised, control group</td>
<td>Static stretching and/or topical heat application</td>
<td>MVC, muscle soreness, T2 relaxation time</td>
<td>No significant effect noted</td>
</tr>
<tr>
<td>Eston et al. (2007)</td>
<td>14</td>
<td>Randomised, control group</td>
<td>PNF stretching performed for 5 weeks prior to EIMD</td>
<td>MVC, muscle soreness, ROM</td>
<td>Recovery of strength at longer muscle lengths in treatment group (p&lt;0.05)</td>
</tr>
<tr>
<td>Citation</td>
<td>N</td>
<td>Design</td>
<td>Intervention</td>
<td>Measures</td>
<td>Results</td>
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<tr>
<td>Smith et al. (1994)</td>
<td>14</td>
<td>Randomised design with control group</td>
<td>30 mins sports massage, 2h post-EIMD</td>
<td>Muscle soreness, CK, neutrophil and cortisol levels</td>
<td>Massage group displayed significantly lower (p&lt;0.05) muscle soreness, CK, neutrophil and cortisol levels</td>
</tr>
<tr>
<td>Zainuddin et al. (2005)</td>
<td>10</td>
<td>Crossover design</td>
<td>10mins sports massage 3h post-EIMD</td>
<td>Muscle soreness, CK, MVC, ROM, limb circumference</td>
<td>Massage group displayed significantly lower (p&lt;0.05) muscle soreness and CK only</td>
</tr>
<tr>
<td>Jönhagen et al. (2004)</td>
<td>16</td>
<td>Randomized</td>
<td>Sports massage given daily for three days post-EIMD</td>
<td>Muscle soreness, vertical jump</td>
<td>No significant effects noted</td>
</tr>
<tr>
<td>Mancinelli et al. (2006)</td>
<td>22</td>
<td>Randomized, control group</td>
<td>Sports massage given post-EIMD</td>
<td>Vertical jump, shuttle run, ROM, muscle soreness</td>
<td>Some significant effects noted in treatment group for vertical jump and muscle soreness (p&lt;0.05)</td>
</tr>
<tr>
<td>Nosaka &amp; Clarkson (1997)</td>
<td>9</td>
<td>Contralateral limb served as control</td>
<td>Concentric exercise performed immediately prior to EIMD</td>
<td>Muscle soreness, MVC, CK, relaxed and flexed</td>
<td>Treatment resulted in lower level of soreness, faster recovery of force</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Design</td>
<td>Intervention Methodology</td>
<td>Outcomes</td>
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<tr>
<td>Zainuddin et al. (2006)</td>
<td>14</td>
<td>Contralateral limb served as control</td>
<td>Light, concentric exercise performed daily for four days following EIMD</td>
<td>MVC, ROM, muscle soreness, circumference, smaller decrease in joint angle and CK (p&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>Saxton and Donnelly (1995)</td>
<td>8</td>
<td>Contralateral limb served as control</td>
<td>Light, concentric exercise performed daily for four days following EIMD</td>
<td>CK, joint angle, MVC, Some effect on indices of muscle soreness (p&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>Kraemer et al. (2001)</td>
<td>20</td>
<td>Randomised, controlled design</td>
<td>Compression garment worn for 5 days following EIMD</td>
<td>1RM, circumference, joint angle, serum cortisol, CK, LDH, muscle soreness, Significant effects noted (p&lt;0.05) for loss of elbow extension, reduced soreness and swelling and more rapid force recovery</td>
<td></td>
</tr>
<tr>
<td>Gill et al. (2006)</td>
<td>12</td>
<td>Randomized, controlled design</td>
<td>Compression sleeve worn for 12 hours following EIMD</td>
<td>CK, Treatment associated with significantly lower CK scores (p&lt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>
Of the numerous interventions proposed to either limit the effects of, or speed the recovery from EIMD, protein ingestion and the RBE have consistently been shown to be effective. Investigations thus far are yet to establish the timings effects of acute protein ingestion, as well as any interactions with the repeated bout effect.

This body of work sets out to test the hypothesis that the ingestion of whey protein hydrolysate will lead to enhanced recovery of muscle function, that a timing effect exists and that the ingestion of WPH prior to eccentric exercise conveys a protective effect, and finally that WPH enhances the RBE.

4. Methodology and Study Design

Ethical approval

Ethical approval was sought prior to any research being conducted. An application was made to the University of Exeter ethical approval committee for all studies in February 2010. The studies outlined as part of the application satisfied all criteria (path B) and ethical approval was granted in March 2010.

Risk assessment

Prior to subject recruitment and data collection, risk assessment of the dietary intervention (WPH), the testing procedures and the muscle-damaging bout of exercise were undertaken. This was in the format of the risk assessment pro-forma from the University of Exeter, and included as part of the ethics application. An example of a completed risk assessment form can be found in Appendix 1.

Nature of supplementation

The studies involved the administration of a dietary supplement that is widely available commercially. No other dietary modifications were required. The supplement contained nutrients that occur naturally in the diet and were administered once, within the recommended servings as set out by the manufacturer. The nutritional supplement has been the focus of previous research (Buckley et al., 2010; Tipton et al.2007), and no complications arising from its ingestion have been noted by these investigators. As part of the
screening process, those with any previous allergic or sensitivity response to dairy proteins were ineligible to take part in the study.

WPH supplementation was in the form of a commercially available powder (Myprotein, Manchester, UK) with a degree of hydrolysis of 13%, mixed with 200ml of water as per the manufacturer’s directions immediately prior to consumption, administered orally at the stated time. Dosages were within the manufacturer’s recommended range and in line with those given in previous studies (25g, Buckley et al., 2010; 100g, Etheridge et al., 2008). Sucrose placebo was in the form of a commercially available powder (Myprotein, Manchester, UK), mixed with 200ml of water. All supplements were prepared by an independent third party at the testing site immediately prior to ingestion. The identification of each supplement was blinded to the investigator.

**Subject recruitment**

Subjects (males aged 18-23 years) were recruited via public advertisement. Due to the nature of the muscle-damaging protocol and associated tests, volunteers were excluded from the study if in the past three months they had undertaken regular (once a week or more) resistance training of the quadriceps muscles; had/have a knee, quadriceps or other musculoskeletal or medical problem which might interfere in their ability to perform the required exercise and tests; had any previous allergic or sensitivity response to dairy proteins, or been diagnosed with liver damage, malnutrition, or a defect of amino acid metabolism.

Prior to participation all subjects received information regarding the studies, completed a health questionnaire and gave written informed consent. Examples of these forms can be found in Appendices 2, 3 and 4.

**Testing procedures**

All subjects undertook two familiarisation sessions. The first consisting of an explanation, demonstration and then a warm-up followed by three opportunities to practice performing each of the measures. The second familiarisation session consisted of a warm-up and three further attempts at each of the performance measures.
Warm-Up

Prior to each bout of testing, subjects completed a warm-up consisting of 5x10m of light jogging and dynamic stretches (2x10m carioca step; 10m walking lunges, alternating legs; 10m heel flicks; 10m high knee jogging).

Squat Jump

Squat jump height was measured using a Globus Ergotester Jump Mat (Cordogne, TV, Italy). Volunteers were instructed to hold a squat position (90 degree hip and knee flexion) for three seconds before jumping as high as possible using an arm swing. Subjects performed three trials separated by 1min of recovery. The best of the three trials was recorded.

Ground Contact Time

Time in contact with the ground was assessed using an Optojump device (Microgate, Bolzano, Italy). Volunteers were instructed to step from a 50cm box, land two-footed and jump immediately over a 12” hurdle placed 50cm in front of the box. Subjects performed three trials separated by 1min of recovery. The best of the three trials was recorded.

Sprint Performance

Sprint performance was assessed using a set of wireless timing gates (Brower, Draper, Utah, USA). For all distances, subjects began from a standing start, 1m behind a set of timing gates. Subjects performed two 10m sprints separated by 2mins of recovery with the best time recorded. Timings were taken for 0-5m, 5-10m and 0-10m. Subjects performed two 20m sprints separated by 2mins of recovery with the best 0-20m time recorded.

Muscle Soreness

Muscle soreness was evaluated using a 100mm visual analogue scale (VAS) which has been used as a valid indicator of pain in previous research (Ohtani et al., 2006; Wojcik wt al., 2001) having obtained reliability scores as high as $r = 0.97$ for assessing soreness (Ohtani et al., 2006), as well as correlating with MVC (Nosaka et al., 2006). The VAS consisted of a horizontal line with anchor points consisting of ‘no soreness’ on the left and ‘worst pain ever experienced’.
on the right. Volunteers were instructed to hold a squat position (90 degrees of hip and knee flexion) for two seconds and return to standing (Howatson et al., 2010; Goodall & Howatson, 2008). The volunteers placed a mark at the point on the VAS corresponding to their perception of the soreness in the quadriceps muscles of the leg. The extent of the muscle soreness was quantified using the measured distance (in mm) from the left hand end of the continuum to the mark made by the volunteer.

**Study design**

All studies used a randomised, double-blind, placebo controlled, parallel design. Group allocation and supplement preparation was conducted by an independent third party.

All data was collected at Sussex Downs College (Eastbourne, East Sussex) by the primary investigator (Kristoph Thompson). This data was stored separately to all participant information thus satisfying all data protection concerns, as well as the double-blind nature of the studies.

**Statistical analysis**

A mixed model repeated measures analysis of variance (ANOVA) was used to determine the effects of supplement timing on the dependent measures over time (SPSS Statistical Analysis for Windows Version 21.0). Post hoc analysis was conducted to identify differences between means using Tukey’s test. Statistical significance was set at an α-level of P<0.05.
5. Study 1 - The effects of whey protein hydrolysate on muscular performance following a bout of eccentric exercise – a pilot study

Abstract

**Purpose:** This purpose of this pilot study was to determine if the acute ingestion of Whey Protein Hydrolysate (WPH) would have an effect on muscle damage, function and soreness. A secondary objective was to identify whether the timing of WPH ingestion would have any effect on muscle function and soreness following EIMD.

**Methods:** Twenty three untrained males (\( \bar{x} \) age = 18.8±0.4y) received a supplement before or after eccentric exercise (ECC). Subjects were randomly allocated to one of four groups; group 1 consuming 200ml of flavoured water (FW) with 25g whey protein hydrolysate immediately pre-ECC (Pro-Pre, n=5); group 2 consuming 200 ml of FW with 25g whey protein hydrolysate immediately post-ECC (Pro-Post, n=4); group 3 consuming 200 ml of FW with 25g sucrose placebo immediately pre-ECC (Plac-Pre, n=7); group 4 consuming 200 ml of FW with 25g sucrose placebo immediately post-ECC (Plac-Post n=7). Subjects performed 100 countermovement jumps in a 10x10 protocol. Measures of sprint, squat and depth jump performance as well as muscle soreness were performed pre, and at 24, 48, and 120h post-exercise. Mixed model repeated measures ANOVA were used to analyse data.

**Results:** Measures of muscle function were significantly reduced as a result of muscle-damaging exercise (P<0.05), returning to baseline values by 120h for all four groups. There were some group by time interactions for sprint performance, squat jump and ground contact time at 24h and 48h, with WPH exerting some effect on the recovery of muscle function as compared to placebo (P<0.05).

**Conclusion:** WPH ingestion appeared to facilitate the enhanced recovery of muscle function as compared to a carbohydrate placebo. There was no significant effect associated with the timing of ingestion (pre or post-EIMD).
Introduction
Dietary protein supplementation has received significant focus in relation to its
ergogenic properties; the first protein supplement marketed as an ergogenic aid
appeared in the 1950’s and initial research in the 1960’s and 70’s examined the
effects of differing protein intakes on body mass, nitrogen balance and athletic
performance (Consolazio et al., 1975; Gontzea et al., 1975; Rasch et al., 1969;
Rasch and Pierson, 1964). This initial research yielded conflicting results
however this may in part be due to the variety of study designs, populations,
and training interventions employed by the various studies. More recent
research (Willoughby et al., 2007; Cadow et al., 2006) has illustrated the
benefits of adequate protein intake and time of ingestion in augmenting the
training effect.

The effect of exercise on protein turnover has been well researched; exercise
has been shown to increase protein catabolism and dietary protein
requirements (Friedman and Lemon, 1989; Meredith et al., 1989; Tarnopolsky
et al., 1988). EIMD results in damage to contractile proteins (Lieber and Fridén,
1999). Protein supplementation increases the availability and utilisation of
branched chain amino acids (BCAA) and has been hypothesised to reduce
catabolism during resistance training (Krieder, 1999, Krieder et al., 1996).
Whey protein ingestion has been found to result in a rapid increase in plasma
levels of amino acids, stimulating protein synthesis and little change in protein
catabolism (Boirie et al., 1997). As a result, these changes in protein synthesis
associated with protein ingestion should reduce markers of EIMD and facilitate
the return of muscle function to baseline levels (Saunders, 2007).

WPH was shown to enhance the recovery of force generating capacity following
a bout of EIMD (Buckley et al., 2010) when a single dose of WPH was
administered immediately after the muscle damaging bout. The effects of acute
WPH administration warrant further research as it is unclear whether the timing
of WPH administration conveys any effect on performance measures following
a bout of EIMD.
The purpose of this study was to investigate the timing effect of WPH ingestion on the recovery of muscle function following a bout of muscle damaging exercise. It is hypothesised that WPH ingestion would result in reduced symptoms of EIMD and loss of functional performance as compared to placebo, supporting the findings of Buckley et al. (2010), and that WPH consumed immediately after exercise would ameliorate the effects of EIMD to a greater extent than when consumed prior to exercise.

Methods
Twenty-three males (\( \bar{x} \text{ age} = 18.8 \pm 0.4y; \text{ height} = 1.79 \pm 0.1m; \text{ mass} = 73.4 \pm 3.4kg \)) were recruited via public advertisement (Table 5.1). The study used a randomised, double-blind, placebo controlled, parallel design. Following familiarisation sessions, measures of sprint, squat and depth jump performance as well as muscle soreness were taken prior to undertaking muscle-damaging exercise. Countermovement jumps have been shown to elicit muscle damage in previous research (Jakeman et al., 2009; Twist and Eston, 2009) and were therefore employed in this study. Subjects performed 100 maximal countermovement jumps in a 10x10 protocol (ECC).

These measures were repeated at 24, 48, and 120 h following ECC. These time points were chosen as they are in line with the generally delayed response of the indicators of muscle damage used within the present study, that peak at 24-48 hours following eccentric exercise, with a return to baseline noted at 120h after the completion of the exercise bout (Avela et al., 1999; Semark et al., 1999; Newham et al., 1988).

Previous work has shown that administration of 25g WPH conveyed a significant effect on markers of muscle damage (Buckley et al., 2010), as well as increasing protein synthesis (Kerksick et al., 2006). Subjects were randomly allocated to one of four groups; group 1 consumed 200ml of flavoured water (FW) with 25 g whey protein hydrolysate immediately pre ECC (Pro-Pre); group 2 consumed 200ml of FW with 25 g whey protein hydrolysate immediately post ECC (Pro-Post); group 3 consumed 200ml of FW with 25 g sucrose placebo immediately pre ECC (Pro-Pre); group 4 consumed 200ml of FW with 25 g sucrose placebo immediately post ECC (Pro-Post).
Volunteers were excluded from the study if in the past three months they had undertaken regular (once a week or more) resistance training of the quadriceps muscles; had/have a knee, quadriceps or other musculoskeletal or medical problem which might interfere in their ability to perform the required exercise and tests; had any previous allergic or sensitivity response to dairy proteins, or been diagnosed with liver damage, malnutrition, or a defect of amino acid metabolism.

Ethics approval was gained from the University of Exeter prior to commencing the study. All volunteers completed written informed consent prior to participation.

Muscle-damaging protocol

Muscle damage was induced using 100 (10x10, 1min rest between sets) maximal countermovement jumps. Subjects lowered to 90 degrees of hip and knee flexion before jumping as high as possible. This was repeated immediately upon landing until ten repetitions had been completed. This protocol has been previously shown to elicit muscle damage (Jakeman et al., 2009; Highton, Twist and Eston, 2009).

Warm-Up

Prior to each bout of testing, subject completed a warm-up consisting of 5x10m of light jogging and dynamic stretches (2x10m carioca step; 10m walking lunges, alternating legs; 10m heel flicks; 10m high knee jogging).

Squat Jump

Squat jump height was measured using a Globus Ergotester Jump Mat (Cordogne, TV, Italy), volunteers were instructed to hold a squat position (90 degree hip and knee flexion) for three seconds before jumping as high as possible using an arm swing. Subjects performed three trials separated by 1min of recovery. The best of the three trials was recorded.

Ground Contact Time

Time in contact with the ground was assessed using an Optojump device (Microgate, Bolzano, Italy). Volunteers were instructed to step from a 50cm
box, land two-footed and jump immediately over a 12” hurdle placed 50cm in front of the box. Subjects performed three trials separated by 1min of recovery. The best of the three trials was recorded.

Sprint Performance

Sprint performance was assessed using a set of wireless timing gates (Brower, Draper, Utah, USA). For all distances, subjects began from a standing start, 1m behind a set of timing gates. Subjects performed two 10m sprints separated by 2mins of recovery with the best time recorded. Timings were taken for 0-5m, 5-10m and 0-10m. Subjects performed two 20m sprints separated by 2mins of recovery with the best 0-20m time recorded.

Muscle Soreness

Muscle soreness was evaluated using a 100mm visual analogue scale (VAS). The VAS consisted of a horizontal line with anchor points consisting of ‘no soreness’ on the left and ‘worst pain ever experienced’ on the right. Volunteers were instructed to hold a squat position (90 degrees of hip and knee flexion) for two seconds and return to standing. The volunteers placed a mark at the point on the VAS corresponding to their perception of the soreness in the quadriceps muscles of the leg. The extent of the muscle soreness was quantified using the measured distance (in mm) from the left hand end of the continuum to the mark made by the volunteer.

Statistical Analysis

Baseline parameters for each group was compared using one-way analysis of variance (ANOVA). Mixed model repeated measures ANOVA was used to determine the effects of supplement timing on the dependent measures over time. Post hoc analysis was conducted to identify differences between means using Tukey’s test. Statistical significance was set at an α-level of P<0.05. All data are shown as mean ±S.E.M.

Table 5.1: Subject Characteristics (n=23)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Pre (n=5)</td>
<td>18.1</td>
<td>1.77 ± 0.08</td>
<td>74.2 ± 3.70</td>
</tr>
</tbody>
</table>
Protein Post (n=4)  18.4 ± 1.14  1.75 ± 0.12  75.8 ± 6.78
Placebo Pre (n=7)  18 ± 0  1.78 ± 0.11  72.6 ± 2.90
Placebo Post (n=7)  18.8 ± 0.65  1.76 ± 0.02  76.7 ± 3.41

Values shown are means ± standard deviation
†significant difference between groups

Results

Table 5.2: Muscle Soreness, VAS, SJ, Sprint Performance, GCT following the muscle damaging protocol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>0</th>
<th>24hrs</th>
<th>48hrs</th>
<th>120hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Analogue</td>
<td>Protein Pre</td>
<td>0.46 ± 0.40</td>
<td>0.9 ± 1.21</td>
<td>1.04 ± 1.55</td>
<td>0.44 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>Protein Post</td>
<td>0.88 ± 1.03</td>
<td>0.91 ± 0.68</td>
<td>1.28 ± 1.21</td>
<td>0.25 ± 0.1</td>
</tr>
<tr>
<td>Scale (cm)</td>
<td>Placebo Pre</td>
<td>0.54 ± 0.73</td>
<td>0.72 ± 1.06</td>
<td>0.67 ± 0.73</td>
<td>0.81 ± 0.97</td>
</tr>
<tr>
<td></td>
<td>Placebo Post</td>
<td>0.59 ± 0.89</td>
<td>1.84 ± 1.8</td>
<td>1.94 ± 2.27</td>
<td>0.43 ± 0.63</td>
</tr>
<tr>
<td>Squat Jump (cm)</td>
<td>Protein Pre</td>
<td>43.9 ± 10.59</td>
<td>41.7 ± 9.92</td>
<td>42.64 ± 10.9</td>
<td>43.96 ± 10.57</td>
</tr>
<tr>
<td></td>
<td>Protein Post</td>
<td>39.13 ± 1.62</td>
<td>37.6 ± 1.85</td>
<td>36.45 ± 1.28</td>
<td>37.95 ± 1.81</td>
</tr>
<tr>
<td></td>
<td>Placebo Pre</td>
<td>45.18 ± 8.44</td>
<td>43.76 ± 8.35</td>
<td>43.7 ± 7.70</td>
<td>43.43 ± 7.60</td>
</tr>
<tr>
<td></td>
<td>Placebo Post</td>
<td>36.91 ± 3.21</td>
<td>35.45 ± 3.10</td>
<td>32.59 ± 3.59*</td>
<td>36.71 ± 4.11</td>
</tr>
<tr>
<td>0-5m Sprint (secs)</td>
<td>Protein Pre</td>
<td>1.13 ± 0.08</td>
<td>1.18 ± 0.08</td>
<td>1.17 ± 0.11</td>
<td>1.12 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Protein Post</td>
<td>1.17 ± 0.07</td>
<td>1.15 ± 0.03</td>
<td>1.20 ± 0.04</td>
<td>1.18 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Placebo Pre</td>
<td>1.13 ± 0.1</td>
<td>1.16 ± 0.09</td>
<td>1.15 ± 0.06</td>
<td>1.14 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Placebo Post</td>
<td>1.19 ± 0.08</td>
<td>1.23 ± 0.09</td>
<td>1.24 ± 0.07</td>
<td>1.2 ± 0.07</td>
</tr>
<tr>
<td>5-10m Sprint (secs)</td>
<td>Protein Pre</td>
<td>0.8 ± 0.04</td>
<td>0.8 ± 0.04</td>
<td>0.77 ± 0.04</td>
<td>0.83 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Protein Post</td>
<td>0.81 ± 0.02</td>
<td>0.79 ± 0.05</td>
<td>0.81 ± 0.03</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Placebo Pre</td>
<td>0.77 ± 0.06</td>
<td>0.81 ± 0.06*</td>
<td>0.79 ± 0.08</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Placebo Post</td>
<td>0.8 ± 0.02</td>
<td>0.84 ± 0.03*</td>
<td>0.82 ± 0.03</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>0-10m Sprint (secs)</td>
<td>Protein Pre</td>
<td>1.94 ± 0.12</td>
<td>2.0 ± 0.09</td>
<td>1.97 ± 0.14</td>
<td>1.94 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Protein Post</td>
<td>2.0 ± 0.04</td>
<td>1.93 ± 0.07</td>
<td>1.99 ± 0.08</td>
<td>2.02 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Placebo Pre</td>
<td>1.93 ± 0.12</td>
<td>1.98 ± 0.12</td>
<td>1.95 ± 0.11</td>
<td>1.97 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Placebo Post</td>
<td>2.03 ± 0.04</td>
<td>2.08 ± 0.11</td>
<td>2.07 ± 0.1</td>
<td>2.08 ± 0.06</td>
</tr>
<tr>
<td>0-20m Sprint</td>
<td>Protein Pre</td>
<td>3.29 ± 0.16</td>
<td>3.32 ± 0.12</td>
<td>3.37 ± 0.08</td>
<td>3.34 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Protein Post</td>
<td>Placebo Pre</td>
<td>Placebo Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(secs) Ground</td>
<td>3.35 ± 0.05</td>
<td>3.32 ± 0.22</td>
<td>3.42 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.35 ± 0.1</td>
<td>3.32 ± 0.23</td>
<td>3.53 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.44 ± 0.16</td>
<td>3.33 ± 0.21</td>
<td>3.54 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.42 ± 0.07</td>
<td>3.34 ± 0.21</td>
<td>3.51 ± 0.08*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protein Pre</th>
<th>Placebo Pre</th>
<th>Placebo Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>(secs) Contact Time</td>
<td>0.22 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.24 ± 0.03</td>
<td>0.21 ± 0.04*</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>0.23 ± 0.02</td>
<td>0.21 ± 0.03</td>
<td>0.23 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>0.22 ± 0.02</td>
<td>0.2 ± 0.02</td>
<td>0.21 ± 0.04</td>
</tr>
</tbody>
</table>

Values shown are means ± standard deviation
*treatment group significantly different to baseline

Muscle Soreness

There was no significant difference between groups at baseline and while some increase in soreness was noted at 24h and 48h there were no significant differences noted in either treatment or control groups.

Squat Jump

There was no significant main effect for time noted. There was some group x time interaction however, with post hoc analysis revealing scores in the Plac-Post group being significantly lower at 48h as compared to baseline (p=0.046). No other significant group differences were noted at this time point.

Sprint Performance

There was no significant main effect for time or group x time interaction for 0-5m. There was a significant group by time interaction noted for 5-10m sprint performance with post-hoc analysis revealing that performance in the Plac-Pre and Plac-Post groups was significantly lower at 24h as compared to baseline (p=.045; p=.045). Performance in 0-20m sprint was significantly lower across all time points for the Plac-Post group (24h, p=.047; 48h, p=.032; 120h, p=.009) as compared to baseline.

Ground Contact Time

There was some group by time interaction with post-hoc analysis revealing that scores were significantly higher for the Plac-Pre group at 24h (p=.04) and Plac-Post group at 48h (p=.02) as compared to baseline.
Discussion
The present study sought to examine if the acute administration of WPH would lead to a reduction in the effects of EIMD following a bout of eccentric exercise designed to bring about muscle damage. Additionally, the study set out to investigate whether any timing effect was noted between treatment groups, indicating a performance advantage in the administration of WPH immediately before or after a bout of strenuous exercise.

Ingestion of WPH appeared to convey some protective effect against a bout of muscle damaging exercise. This was as evidenced by significantly worsened scores in performance measures (sprint performance, squat jump and ground contact time) at 24h and 48h amongst those subjects not consuming WPH, as compared to those groups consuming WPH.

These findings are in concurrence with those of previous work (Buckley et al., 2010; Etheridge et al., 2008) that have shown the protective effects associated with WPH ingestion. If WPH were to result in any protective effect or enhanced recovery, it would only extend to measures of muscle performance and not muscle soreness.

It would appear that there is no timing effect associated with any protective effects, or the recovery of muscle function associated with acute WPH ingestion following 100 CMJs.

Conclusion

Although the acute ingestion of WPH may have some effect on the recovery of muscle function following a bout of muscle-damaging exercise, the timing of this dose of WPH appears to have no significant effect. The findings may have some direct application to those engaged within sports or activities where sprint and/or jump performance is linked to overall performance.
6. Study 2: Timing effects of whey protein hydrolysate on muscular performance following a bout of eccentric exercise

Abstract

**Purpose:** To determine if timing of Whey Protein Hydrolysate (WPH) ingestion would have an effect on muscle damage, function and soreness.

**Methods:** Twenty untrained males (18.5 ± 0.6 y) received a supplement before or after eccentric exercise (ECC). Subjects were randomly allocated to one of four groups: group 1 consuming 200ml of flavoured water (FW) with 25g whey protein hydrolysate immediately pre-ECC (Pro-Pre, n=5); group 2 consuming 200 ml of FW with 25 g whey protein hydrolysate immediately post-ECC (Pro-Post, n=5); group 3 consuming 200 ml of FW with 25 g sucrose placebo immediately pre-ECC (Plac-Pre, n=5); group 4 consuming 200 ml of FW with 25 g sucrose placebo immediately post-ECC (Plac-Post n=5). Subjects performed 100 eccentric leg extensions at 80% of eccentric 1RM, each lasting 5 s in a 10x10 protocol. Measures of sprint, squat and depth jump performance as well as muscle soreness were performed pre- and again at 24, 48, and 120h post-exercise. Mixed model ANOVA was used to analyse data.

**Results:** Muscle soreness increased and muscle function were significantly reduced as a result of muscle-damaging exercise, as compared to baseline values. WPH exerted some effect on the recovery of muscle function at 48h as compared to placebo. 5-10m sprint speed was significantly higher at 48h amongst those that received the placebo (p<0.01 & p<0.006), as compared to those receiving WPH.

**Conclusion:** Ingestion of an acute dose of WPH may convey facilitate the recovery of muscle function (sprint speed) at 48h, therefore may have some application to those engaged in activities where sprint performance impacts overall sporting performance.
Introduction

Symptoms associated with EIMD include soreness, tenderness, changes in range of motion, strength loss, and release of muscle proteins such as creatine kinase (Eston, Mickleborough & Baltzopoulos, 1995). All of these result in impaired muscle function of the local area and performance in tasks such as squat jump and sprints (Byrne and Eston, 2002b; Clarkson & Hubal, 2002; McHugh et al., 1999b; Clarkson, Nosaka & Braun 1992; Byrnes et al., 1985; Armstrong, 1984).

Whey protein hydrolysate (WPH) has been shown to enhance protein synthesis (Tang et al., 2009). It is absorbed at a faster rate from the small intestine than whole milk proteins delivered as a milk solution (Buckley et al., 2010), and can accelerate recovery of muscle function following a bout of muscle damage inducing exercise (Buckley et al., 2010; Etheridge, Philp & Watt, 2008).

Amino acids are thought to exert their protective effects through a combination of direct and indirect mechanisms. Directly, amino acids may depress pathways responsible for Z-Line disruption during the metabolic cascade triggered by mechanical trauma (Helman et al., 2003; Belcastro, Shewchuk & Raj 1998) while indirect mechanisms relate to formation of specific amino acid derived metabolites, such as Beta-Hydroxy-Beta-Methylbutyrate, shown in studies to lower indices of muscle damage (Nissen et al., 1996).

Whilst there appears to be some evidence supporting the acute administration of WPH (Buckley et al., 2010; Etheridge, Phil & Watt, 2008) in ameliorating the effects of EIMD, the timing effect of hydrolysate ingestion on performance measures following a bout of EIMD is currently unknown. Study 1 within this body of work sought to examine whether any the timing of WPH administration conveyed any effects on muscle function. The extent of muscle damage conveyed was relatively small; <5% as compared to 15% reduction in squat jump performance (Byrne & Eston, 2002b). The purpose of this study was to investigate the timing effect of WPH ingestion on the recovery of muscle function following a bout of muscle damaging exercise where an alternative muscle damaging protocol was employed.
Methods

Twenty males (\(\bar{\text{age}} = 18.5 \pm 0.6\text{y}; \text{height} = 1.76 \pm 0.05\text{m}; \text{mass} = 75.05 \pm 4.69\text{kg}\)) were recruited by public advertisement. The study used a randomised, double-blind, placebo controlled, parallel design. Baseline measures of sprint, squat and depth jump performance as well as muscle soreness were taken prior to undertaking 100 eccentric leg extensions (ECC) at 80% of eccentric 1RM, each lasting 5 s in a 10x10 protocol. These measures were repeated at 24, 48, and 120 h following ECC. Subjects were randomly allocated to one of four groups; group 1 consumed 200ml of flavoured water (FW) with 25 g whey protein hydrolysate immediately pre ECC (Pro-Pre); group 2 consumed 200ml of FW with 25 g whey protein hydrolysate immediately post ECC (Pro-Post); group 3 consumed 200ml of FW with 25 g sucrose placebo immediately pre ECC (Pro-Pre); group 4 consumed 200ml of FW with 25 g sucrose placebo immediately post ECC (Pro-Post).

Volunteers were excluded from the study if in the past three months they had undertaken regular (once a week or more) resistance training of the quadriceps muscles; had/have a knee, quadriceps or other musculoskeletal or medical problem which might interfere in their ability to perform the required exercise and tests; had any previous allergic or sensitivity response to dairy proteins, or been diagnosed with liver damage, malnutrition, or a defect of amino acid metabolism.

Ethics approval was gained from the University of Exeter prior to commencing the study. All volunteers completed written informed consent prior to participation.

Muscle-damaging protocol

Muscle fatigue and muscle damage was induced using 100 (10x10, 1min rest between sets) eccentric leg extensions, each lasting 5 s at 80% of eccentric 1RM using a seated leg extension machine adjusted for each individual (ST750, Vision Fitness, Stoke-On-Trent, Staffordshire, UK). This protocol has been previously employed and shown to be effective in eliciting muscle damage (Jamurtas et al., 2005; Paschalis et al., 2005). Subjects began each movement with both knees extended at 180 degrees, lowering both to a count of five to 90
degrees of flexion. The knee extensors were relaxed at the end of each eccentric phase and the relaxed legs were returned to the starting position by the experimenter.

Warm-Up

Prior to each bout of testing, subject completed a warm-up consisting of 5x10m of light jogging and dynamic stretches (2x10m carioca step; 10m walking lunges, alternating legs; 10m heel flicks; 10m high knee jogging).

Squat Jump

Squat jump height was measured using a Globus Ergotester Jump Mat (Cordogne, TV, Italy) which measures flight time and in turn calculates vertical jump height. This device has previously been shown to be a valid and reliable means of assessing vertical jump (ICC = 0.972 – 0.990, (Santos-Lozano et al., 2014)). Volunteers were instructed to hold a squat position (90 degree hip and knee flexion) for three seconds before jumping as high as possible using an arm swing. Subjects performed three trials separated by 1min of recovery. The best of the three trials was recorded.

Ground Contact Time

Time in contact with the ground was assessed using an Optojump device (Microgate, Bolzano, Italy). This device has previously been shown to be a valid and reliable tool with intraclass correlation coefficients of 0.997-0.998 and test-retest reliability ICC’s ranging from 0.982-0.989 (Glatthorn et al., 2011). Volunteers were instructed to step from a 50cm box, land two-footed and jump immediately over a 12” hurdle placed 50cm in front of the box. Subjects performed three trials separated by 1min of recovery. The best of the three trials was recorded.

Sprint Performance

Sprint performance was assessed using a set of wireless timing gates (Brower, Draper, Utah, USA). For all distances, subjects began from a standing start, 1m behind a set of timing gates. Subjects performed two 10m sprints separated by 2mins of recovery with the best time recorded. Timings were taken for 0-5m, 5-
10m and 0–10m. Subjects performed two 20m sprints separated by 2mins of recovery with the best 0–20m time recorded.

Muscle Soreness

Muscle soreness was evaluated using a 100mm visual analogue scale (VAS). The VAS consisted of a horizontal line with anchor points consisting of ‘no soreness’ on the left and ‘worst pain ever experienced’ on the right. Volunteers were instructed to hold a squat position (90 degrees of hip and knee flexion) for two seconds and return to standing. The volunteers placed a mark at the point on the VAS corresponding to their perception of the soreness in the quadriceps muscles of the leg. The extent of the muscle soreness was quantified using the measured distance (in mm) from the left hand end of the continuum to the mark made by the volunteer.

Statistical Analysis

Baseline parameters for each group was compared using one-way analysis of variance (ANOVA). Mixed model repeated measures ANOVA was used to determine the effects of supplement timing on the dependent measures over time. Post hoc analysis was conducted to identify differences between means using Tukey’s test. Statistical significance was set at an α-level of P<0.05. All data are shown as mean ± S.E.M.

Table 6.1: Subject Characteristics (n=20)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Pre (n=5)</td>
<td>18.4</td>
<td>1.79 ± 0.06</td>
<td>78.2 ± 3.70</td>
</tr>
<tr>
<td>Protein Post (n=5)</td>
<td>19.4 ± 1.14</td>
<td>1.73 ± 0.02</td>
<td>75.4 ± 7.44</td>
</tr>
<tr>
<td>Placebo Pre (n=5)</td>
<td>18 ± 0</td>
<td>1.77 ± 0.09</td>
<td>70.4 ± 3.85</td>
</tr>
<tr>
<td>Placebo Post (n=5)</td>
<td>18.2 ± 0.45</td>
<td>1.74 ± 0.02</td>
<td>76.2 ± 3.77</td>
</tr>
</tbody>
</table>

Values shown are means ± standard deviation
Results

Muscle Soreness

There was no difference in muscle soreness between groups at baseline (P=0.53). As compared to baseline, muscle soreness increased in all groups at 24h and 48h, returning to baseline values at 120h. There was no significant group x time interaction on muscle soreness (F(9, 48) = 1.99, P=0.061). There was a main effect for time (F(3,48) = 17.64). P<0.01). Post Hoc analysis showed that muscle soreness scores were significantly elevated at 24h (p<0.01) and 48h (p =<0.01) as compared to baseline.

Figure 6.1: Changes in muscle Soreness (VAS) after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different (p<0.05) from baseline.

Squat Jump

There was no difference in squat jump between groups at baseline (P=0.595) but squat jump scores were significantly reduced at 24h and 48h as compared to baseline (p<0.01). There was no group by time interaction (F (4.83, 25.76) = 2.10, P=0.100).
Figure 6.2: Changes in Squat Jump after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.) * Significantly different (p<0.05) from baseline.

Ground Contact Time (GCT)

As shown in figure 6.3, ground contact time increased from baseline values across all groups, peaking at 24h and returning to baseline values by 120h. There was a main effect for time (F (1.42, 22.641) = 16.05, p<0.01) with values significantly higher than baseline for all groups at 24h (p<0.001) and 48h (p<0.001). There was no group by time interaction (F (4.23, 22.64) = 0.76, P=.569) or main effect for group (F (3, 16) = 1.26, p=0.323).
Figure 6.3: Changes in Ground Contact Time after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.) *
Significantly different (p<0.05) from baseline.

Sprint Performance

0-5m

There was a main effect for time with scores significantly elevated as compared to baseline at 24h (F (1.7, 3) = 63.43, p<0.01) however there was no group x time interaction.
Figure 6.4: Changes in 0-5m sprint time after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different (p<0.05) from baseline.

5-10m

There was a main effect for time (F (1.75, 3) = 87.93, p=<0.01), with a significant group x time interaction (F (5.18, 27.61) = 2.68, p=0.04). The most striking differences between groups were evident between 24 and 48hrs. Post-hoc analysis revealed no significant differences between treatment groups at either 24h (p=0.887) or 48h (p=0.761), indicating no timing effect of WPH. As shown in figure 6.6, no significant difference was noted between Pro-Pre (p=0.061) or Pro-Post (p=0.368) between the two time points, however both placebo groups significantly increased 5-10m sprint times between these time points (Plac-Pre p=0.01; Plac-Post p=0.006). This indicates that WPH may have in some way protected against further decrements in sprint performance between 24 and 48h post-eccentric exercise.
Figure 6.5: Changes in 5-10m sprint time after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)
* Significantly different (p<0.05) from baseline.

Figure 6.6: Changes in 5-10m sprint time after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed % change in mean scores (±S.E.M.)
* Significantly different (p<0.05) from baseline.
0-10m

There was a main effect for time with scores significantly elevated for all groups at 24h as compared to baseline ($F(2.15, 3) = 97.65, p<0.01$). There was no group x time interaction ($F(6.46, 34.44) = 1.28, p=0.289$).

![Graph showing changes in 0-10m sprint time](image)

Figure 6.7: Changes in 0-10m sprint time after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)
* Significantly different ($p<0.05$) from baseline.

0-20m

As shown in figure 6.8, there was a main effect for time with scores significantly elevated at 24h as compared to baseline ($F(1.47, 3) = 49.53, p<0.01$) however there was no group x time interaction ($F(4.41, 23.55) = 1.97, p=0.13$).
Discussion

This study set out to determine whether the timing of ingestion of a WPH supplement influenced the extent of EIMD. Specifically, we compared the symptoms of EIMD when WPH was ingested immediately prior to eccentric resistance exercise or immediately following eccentric resistance exercise. Muscle damage was inferred through squat jump and sprint performance ground contact time, and muscle soreness through the use of a VAS. This study found that there were no differences in markers of muscle damage or soreness between the timing of the protein hydrolysate supplement. There was a group x time interaction between 5 and 10m, indicating that ingestion of protein hydrolysate had some effect on sprint speed, as compared to the placebo. No significant difference was noted between treatment groups.

Muscle Soreness

The exact mechanism responsible for muscle soreness has yet to be fully defined but it is generally accepted that it is associated with the events following mechanical perturbations (Tipton et al., 2004). Pain serves to protect by signalling injury to the tissues. However, the pain associated with EIMD initiates...
between 8 and 24 hours post-exercise, with peak levels occurring at around 24-72 hours (Newham et al., 1988; Bobbert et al., 1986). Since the soreness appears some time after the activity, it presumably does not function to prevent overuse during the activity bout in which the injury occurs (Bobbert et al., 1986). In the present study, muscle soreness increased above baseline for all groups for time points 24h and 48h, returning to baseline values by 120h, with the supplement timing having no effect on soreness scores.

Previous studies, employing a range of methods to induce damage, have reported increases in muscle soreness much greater than those elicited from the muscle damaging protocol used in the present study (Davies et al., 2008; Marginson et al., 2005, Rowlands et al., 2001). Scores of muscle soreness, as measured by VAS, amongst control groups have been reported by increase by a factor of 12-20 after 24h. The muscle damaging protocol in the present study resulted in a threefold increase in ratings of muscle soreness. It is possible that the protocol was not severe enough to elicit damage and that the effect of WPH was masked by the lack of substantial changes in muscle function as a result.

The absence of a timing effect supports the results of the work by White and colleagues (White et al., 2008) who noted no difference in muscle soreness with the ingestion of protein either before or after exercise. These findings are also in line with those of Shimomura et al. (2006) who reported a reduction in muscle soreness following the ingestion of 5g of BCAA prior to exercise, yet previous work examining the effects of WPH (Buckley et al., 2010; Etheridge et al., 2008) showed no changes in muscle soreness across the study period. The mechanism responsible for the reduction in soreness at 48h is unclear, further research into this area is needed to elucidate the means by which protein ingestion may reduce the muscle soreness experienced on the days following eccentric exercise.

Squat Jump

Squat Jump performance has been shown to correlate with plasma, functional, and reported indices of muscle damage, supporting its validity as an indirect measure of muscle damage (Jakeman, Byrne & Eston, 2010; Garcia-Lopez et al., 2006; Byrne & Eston, 2002). In the present study, SJ was significantly lower
at 24 and 48h time points for all groups as compared to baseline values. This would suggest that the muscle damaging protocol employed in the present study resulted in appreciable damage, addressing one of the limitations of the pilot study. No significant timing effect was associated with WPH administration. These findings are in line with those of White and colleagues (2008), who showed no timing effects of a combination of protein and carbohydrate on muscular strength and soreness.

Ground Contact Time

Ground contact time increased significantly (p<0.05) at 24h and 48h as compared to baseline for all groups, returning to baseline levels at 120h. The time frame and magnitude of damage is in line with previous research (Byrne & Eston 2002b; Avela et al., 1999) investigating the effects of EIMD on ground contact time following a drop jump.

When the two treatment and control groups were combined to form one treatment and one control group, there appeared to be some evidence to suggest that the ingestion of WPH either immediately before or after a muscle-damaging bout of exercise does have some effect on the restoration of muscle function. There was no significant difference between groups at baseline (p=0.162), with the bout of damaging exercise significantly increasing GCT in both treatment (p=0.016) and control (p=0.02) groups at 24H. However at 48H GCT in the control group was still significantly elevated (p=.0.04) whereas GCT in the treatment group was not significantly elevated (p=0.127), returning to values approaching that of baseline.

The effect of EIMD is less pronounced in vertical jumps that incorporate an active pre-stretch (Byrne & Eston, 2002b), which enhances the final muscle action. Byrne and Eston (2002b) suggest that one or more of the mechanisms proposed by Van Ingen Schenau et al. (1997) attenuate the detrimental performance effects of exercise-induced muscle damage. Whether WPIH might serve to enhance these mechanisms is unclear. It is also possible that WPIH may facilitate the recovery of excitation-contraction coupling first suggested by Edwards et al. (1977) as a theory to explain the loss of strength associated with EIMD. Further research is needed to examine the exact mechanisms by which
WPH may facilitate the recovery of muscle function following muscle-damaging exercise.

Sprint Performance

The ingestion of WPH appeared to offer some protective effect, as noted by the return to sprint times towards baseline values at 48h for those receiving WPH. These findings are in line with those of the pilot study, as well as Etheridge and colleagues (2008) and Buckley et al. (2010).

No group by time interactions were evident, suggesting that timing of supplement ingestion had no effect on sprint performance measures. There was however some evidence to suggest that ingestion of WPH might limit further decrements in performance noted between 24h and 48h. Twist & Eston (2005) and Highton et al. (2009) have previously investigated the effects of muscle damage on sprint performance. Both of these investigations have shown a reduction in sprint performance at 24h and 48h following a muscle damaging bout of exercise. Interestingly, in the present study, 5-10m sprint performance was significantly reduced (p<0.05) for the both placebo groups (Plac-Pre p=0.01; Plac-Post p=0.006) from time points 24h and 48h, whilst no significant differences were noted between treatment groups between these same time points.

The exact mechanism responsible for reductions in sprint performance is unclear, Twist & Eston (2005) suggested that a reduced reflex sensitivity during the stretch-shortening cycle (SSC) may impair the ability to utilise ground impact forces, thus producing less force during the propulsive phase of the leg movement phase and increasing contact time with the ground. It is possible that an acute dose of WPH might in some way facilitate the recovery of SSC reflex sensitivity, therefore resulting in a more rapid recovery of muscle function. Previous studies (Chen et al., 2007; Dutto & Braun, 2004) have shown a reduction in force production, stride length and stride frequency in sub-maximal running following EIMD. However, it is still unclear whether these kinematic changes are evident during sprint running performance, further research is needed to investigate whether the ingestion of WPIH has any effect on these mechanisms.
Conclusion

The present study showed that there was no significant difference in the effect conveyed by the ingestion of WPH either immediately prior to, or immediately following a bout of EIMD. While there was some evidence to suggest that the acute ingestion of WPH was associated with the restoration of muscle function towards baseline levels as compared to a placebo, the effects did not extend to all of the markers of muscle function examined.

This finding is in line with a recent body of research that has shown the ingestion of supplementary protein conveys no additional benefits when dietary protein needs are met (Phillips and Van Loon, 2011). Whilst the present study did not measure subjects’ protein intake, it is likely that their protein needs were met from their daily diet based on the ranges shown to be sufficient to maintain lean body mass during periods of training. These ranges spanned from 0.37g/lb/body mass (Tarnopolsky et al., 1988) to 0.82g/lb/body mass (Phillips and Van Loon, 2011), even during periods of negative energy balance. This would equate to a requirement of 59 – 131g/day based on the mean body mass of those subjects in the present study. Research has shown the average daily protein intake for UK adults is 91g/day (Halkjaer et al., 2009), well within the accepted range. It is therefore likely that the 25h WPH provided in the present study did not serve to provide any additional benefits since participants were not restricted in their daily dietary protein consumption, nor was their daily energy intake restricted.

If acute WPH ingestion were to convey and positive effects on EIMD, the absence of any timing effect surrounding the administration of WPH (pre- or post-EIMD) could be related to the duration of the muscle damaging bout. The eccentric bout of exercise in the present study lasted approximately 18 minutes which may not have been sufficient to allow for the digestion of whole proteins in order for a significant protective effect to be detected between treatment groups, despite the increased rate of gastric emptying and absorption rate of hydrolysates (Calbet and MacLean, 2002). The eccentric bout of muscle damaging exercise employed by White and colleagues (2008) was similar to the present study, lasting <10mins.
If WPH were indeed to exert any positive influence on markers of muscle
damage associated with EIMD, and a timing effect were to exist, it may become
apparent during muscle-damaging protocols of an extended duration. This may
be an avenue for future research.
7. Study 3: The Effects of Whey Protein Hydrolysate on the Repeated Bout Effect

Abstract

Purpose: To determine if the acute administration Whey Protein Hydrolysate (WPH) would have an effect on muscle damage, function and soreness; impacting upon the repeated bout effect phenomenon following a repeated bout of muscle damaging exercise.

Methods: Ten untrained males (18.1 ± 0.23 y) received a WPH supplement immediately after a repeated bout of eccentric exercise (ECC). Subjects were randomly allocated to one of two groups; group 1 consuming 200ml of flavoured water (FW) with 25g sucrose placebo immediately post-ECC (control, n=5); group 2 consuming 200 ml of FW with 25g whey protein hydrolysate immediately post-ECC (treatment, n=5). Subjects performed 100 eccentric leg extensions at 80% of eccentric 1RM, each lasting 5s in a 10x10 protocol. Measures of sprint, squat and depth jump performance as well as muscle soreness were taken at baseline and then repeated at 24h, 48h, and 120h post-exercise. A mixed model ANOVA was used to analyse data. Post hoc analysis was conducted to identify differences between means using Tukey’s test. Statistical significance was set at an α-level of P<0.05.

Results: Muscle soreness increased and muscle function was significantly reduced as a result of muscle-damaging exercise. 0-10m sprint scores among those given WPH were not significantly elevated at 48h, returning to baseline levels at this point. This may indicate that WPH exerted some effect on the recovery of muscle function at 48h as compared to a flavoured water placebo.

Conclusion: Eccentric exercise caused significant loss of muscle function and increased soreness during the initial and repeated bouts. The effects were less pronounced following the repeated bout, highlighting the presence of the repeated bout effect illustrated by previous research. The ingestion of WPH did facilitate the recovery of sprint speed at 48h but had no effect on any other measures of performance or muscle soreness. These findings would
corroborate those of the previous two studies within the present body of work, that acute WPH administration does not serve to reduce the symptoms associated with EIMD or facilitate the restoration of muscle function.
Introduction

The concept of EIMD has been outlined previously in this body of work, as have those interventions proposed to either minimise the immediate effects of the damaging bout of activity, or to facilitate the recovery of muscle function post-exercise. The present study sought to explore the interactions between two such interventions; the administration of WPH and the repeated bout effect.

Of the many interventions examined or proposed to ameliorate the effects of EIMD, the Repeated Bout Effect (RBE) seems to be well supported by the literature. A prior bout of eccentric exercise would appear to provide a protective effect on indices of damage; CK, muscle soreness and function (Mair et al., 1995; Newham et al., 1987; Byrnes et al., 1985).

The previous findings associated with WPH administration have been addressed within previous chapters however its interaction with the repeated bout effect is presently unknown.

Hypothesis

This study will test the hypothesis that WPH supplementation will enhance the RBE, resulting in less of a decrement in muscle function and performance. In order for WPH to enhance the effects of the RB it must exert some influence over one or more of the mechanisms proposed to explain the RBE. The findings outlined above would suggest that the anabolic effect of protein and the ability of WPH to enhance the recovery of muscle function following EIMD would be based upon cellular adaptations and the potential greater manufacture of contractile proteins. It is considered that WPH would enhance the RBE by increasing the cellular adaptations associated with the RBE.

Methods

Ten males aged 18-22 (\(\bar{x}\) 18± 0.45yrs, 176± 0.06cm, 73.3± 3.81kg) were recruited by public advertisement. This study used a double-blind, randomised, placebo controlled design, examining the effects of WPH on the RBE. All
subjects completed 2 bouts of damage inducing exercise, an initial and repeated bout, separated by 21 days. Subjects were randomly assigned to a treatment or control group and received either:

1. Control 25g sucrose placebo immediately post the repeated bout
2. Treatment 25g WPH immediately post the repeated bout

Baseline measures of squat jump (SJ), time in contact with the ground following drop jump (60cm), sprint performance (20m), acceleration (5-10m), and muscle soreness (Visual Analogue Scale - VAS) were recorded. Subjects took part in two identical bouts of muscle-damaging exercise, separated by 21 days. Measures of squat Jump (SJ), time in contact with the ground following drop jump (50cm), sprint performance (10m & 20m), acceleration (5-10m), and muscle soreness (VAS) would be taken at 24h, 48h, 72h, and 120h after the second bout of muscle-damaging exercise.

Volunteers were excluded from the study if in the past three months they had undertaken regular (once a week or more) resistance training of the quadriceps muscles; had/have a knee, quadriceps or other musculoskeletal or medical problem which might interfere in their ability to perform the required exercise and tests; had any previous allergic or sensitivity response to dairy proteins, or been diagnosed with liver damage, malnutrition, or a defect of amino acid metabolism.

Ethics approval was gained from the University of Exeter prior to commencing the study. All volunteers completed written informed consent prior to participation.

Muscle muscle damage was induced using 100 (10x10, 1min rest between sets) eccentric leg extensions, each lasting 5secs at 80% of eccentric 1RM using a seated leg extension machine adjusted for each individual (ST750, Vision Fitness, Stoke-On-Trent, Staffordshire, UK). The knee extensors were relaxed at the end of each eccentric phase and the relaxed leg was returned to the starting position by the experimenter.
Squat jump height was measured using a Globus Ergotester Jump Mat (Cordogne, TV, Italy). Volunteers were instructed to hold a squat position (90 degree hip and knee flexion) for three seconds before jumping as high as possible using an arm swing. Time in contact with the ground was assessed using an Optojump device (Microgate, Bolzano, Italy). Volunteers were instructed to step from a 50cm box, land two-footed and jump immediately over a 12” hurdle. Sprint performance was assessed using a set of wireless timing gates (Brower, Draper, Utah, USA). Muscle soreness was evaluated using a 100mm visual analogue scale (VAS). The VAS consisted of a horizontal line with anchor points consisting of ‘no soreness’ on the left and ‘worst pain ever experienced’ on the right. Volunteers were instructed to hold a squat position for two seconds and return to standing. The volunteers placed a mark at the point on the VAS corresponding to their perception of the soreness in the quadriceps muscles of the leg. The extent of the muscle soreness was quantified using the measured distance (in mm) from the left hand end of the continuum to the mark made by the volunteer. The validity and reliability of these measures has been previously addressed.

Statistical Analysis

Baseline parameters for each group was compared using one-way analysis of variance (ANOVA). A mixed model ANOVA was used to determine the effects of supplement ingestion on the performance related markers of muscle damage. Post hoc analysis was conducted to identify differences between means using Tukey’s test. Statistical significance was set at an α-level of P<0.05. All data are shown as mean ±S.E.M.

Table 7.1: Subject Characteristic (n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
</tr>
</thead>
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<tr>
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<tr>
<td>2. Protein Post</td>
<td>18.2 ± 0.45</td>
<td>1.74 ± 0.02</td>
<td>76.2 ± 3.77</td>
</tr>
</tbody>
</table>

Values shown are means ± standard deviation
Results

Muscle Soreness

During the initial and repeated bouts muscle soreness increased for both groups at 24 and 48h as compared to baseline, returning to baseline values by 120h. As compared to baseline, ratings of soreness were significantly higher at 24h for group 1 during the initial ($P=0.042$) and repeated ($P=0.004$) bout, and at 48h for both bouts (initial $P=0.046$ and repeated $P=0.015$). The same was true of group 2 with muscle soreness increasing significantly above baseline values for both the initial ($P=0.021$) and repeated bouts ($P=0.004$). Soreness remained significantly elevated above baseline at 48h during both the initial ($P=0.03$) and the repeated bouts ($P=0.01$). No significant differences were noted between groups at any time period across the two bouts.

Figure 7.1: Changes in Muscle Soreness (VAS) after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different ($p<0.05$) from baseline.

Squat Jump

As is evident from figure 7.2, muscular power declined following both bouts of eccentric exercise, remaining depressed at 24 and 48h before returning to baseline levels at 120h. Mean scores for all groups and bouts were significantly reduced at 24h and 48h as compared to baseline ($p<0.05$). Following the repeated bout of eccentric exercise, mean scores across both groups were
significantly higher at 24h and 48h as compared to the initial bout (p=0.04), indicating the presence of some repeated bout effect.

Figure 7.2: Changes in Squat Jump after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)
* Significantly different (p<0.05) from baseline.

Ground Contact Time

There was a main effect for time [Wilks’ Lambda = .15, F(3,6)= 11.41, P=.007, multivariate partial eta squared =.85] with GCT increasing significantly across all groups at 24h and 48h as compared to baseline. Scores returned to baseline values at 120h. The was a significant effect for bout noted [Wilks’ Lambda =.49, F(3,6)= 8.21, P=0.02 multivariate partial eta squared =.51], with the decrement in performance following the repeated bout being less pronounced than the initial bout at 24h and 48h, demonstrating the repeated bout effect shown in previous research. There was no group x time or group x time x bout interactions.
Figure 7.3: Changes in Ground Contact Time (GCT) after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different (p<0.05) from baseline.

Sprint Performance

As can be seen in figures 7.4 – 7.7, sprint performance declined across all groups at 24h and 48h with scores being significantly lower than baseline (p<0.05). The repeated bout effect was evident in 0-10m (p=0.002) and 0-20m (p=0.007) sprint performance at 24h and 48h (p=0.04). There was no significant interactions associated with the ingestion of WPH.

Figure 7.4: Changes in 0-5m sprint performance after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different (p<0.05) from baseline.
Figure 7.5: Changes in 5-10m sprint performance after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different (p<0.05) from baseline.

Figure 7.6: Changes in 0-10m sprint performance after exercise-induced muscle damage, measured at 24h, 48h and 120h and expressed as mean scores (±S.E.M.)

* Significantly different (p<0.05) from baseline.
Discussion

This study set out to determine whether the ingestion of a protein hydrolysate supplement influenced the extent of EIMD following a repeated bout of eccentric exercise. Specifically, it compared the symptoms of EIMD when WPH was ingested immediately prior to a bout of eccentric resistance exercise, performed 21 days after an initial bout. Muscle damage was inferred through squat jump, sprint performance, ground contact time, and muscle soreness through the use of a VAS.

Results showed the presence of a RBE, however WPH ingestion was found not to exert any effect on a repeated bout of eccentric exercise. The present findings showing the presence of a RBE are in line with previous research Mair et al., 1995; Newham et al., 1987; Byrnes et al., 1985).

McHugh and colleagues' (1999a) review of the possible mechanisms behind the RBE posited that the adaptation was a result of neural, connective tissue or cellular adaptations. However, other possible mechanisms include adaptation in excitation-contraction coupling or adaptation in the inflammatory response.
McHugh et al. (1999b) concluded it is unlikely that one theory can explain all of the observations of the repeated bout effect found in the literature. The RBE occurs in electrically stimulated contractions in an animal model, precluding neural adaptation exclusively. Connective tissue and cellular adaptations are unlikely explanations when the repeated bout effect is demonstrated prior to full recovery, and when the fact that the initial bout does not have to cause appreciable damage in order to provide a protective effect is considered. McHugh and colleagues (1999b) stated that the repeated bout effect occurs through the interaction of various neural, connective tissue and cellular factors that are dependent on the particulars of the eccentric exercise bout and the specific muscle groups involved.

More recently, McHugh (2003) conducted a follow-up review outlining advances in the potential mechanisms behind the RBE. It was stated that although there is some evidence to suggest that the RBE is associated with a shift toward greater recruitment of slow twitch motor units, it is more likely that a peripheral, non-neural adaptation predominates since the RBE has been demonstrated with electrically stimulated contractions. With respect to mechanical adaptations there is evidence that both dynamic and passive muscle stiffness increase with eccentric training but there are no studies on passive or dynamic stiffness adaptations to a single eccentric bout. It is suggested that the role of the cytoskeleton in regulating dynamic stiffness is a possible area for future research. With respect to cellular adaptations there is evidence of longitudinal addition of sarcomeres and adaptations in the inflammatory response following an initial bout of eccentric exercise. Inflammatory adaptations are thought to limit the proliferation of damage that typically occurs in the days following eccentric exercise. A unified theory explaining the mechanism or mechanisms for this protective adaptation therefore remains elusive.
Muscle Soreness

Soreness was present following both bouts of exercise, with scores significantly elevated at 24h and 48h and then returning to baseline by 120h. The exact mechanism responsible for muscle soreness has yet to be fully defined however it is accepted that it is secondary to the initial damage that occurs and serves to protect by signalling injury to the tissues (Tipton et al., 2007). However, the pain associated with EIMD initiates between 8h and 24h post-exercise, with peak levels occurring at around 24-72h (Newham et al., 1988; Bobbert et al., 1986). Since the soreness appears sometime after the activity, it presumably does not function to prevent overuse during the activity bout in which the injury occurs (Bobbert et al., 1986).

Squat Jump

Squat Jump performance has been shown to correlate with plasma, functional, and reported indices of muscle damage, supporting its validity as an indirect measure of muscle damage (Jakeman et al., 2010; Garcia-Lopez et al., 2006; Byrne & Eston, 2002b). In the present study, SJ was lower at 24h and 48h for all groups as compared to baseline values, however no significant effect was associated with protein ingestion. In line with the present study, White and colleagues (2008) have previously shown that protein ingestion immediately following a bout of muscle-damaging exercise had no effect on muscular soreness following a bout of damaging exercise.

Previous work has shown that chronic BCAA ingestion may exert a beneficial effect on markers if EIMD (Ohtani et al., 2002; Coombes et al., 2000). In this instance, BCAA’s were provided in addition to subjects’ normal diet (which analysis showed to already meet the RDA of BCAA). Therefore, while there may be some beneficial effects to be seen via the ingestion of supplementary BCAA’s, their actions are either as a result of the cumulative effects (as opposed to acute ingestion) or as a result of mechanisms unrelated to acute protein ingestion.
Ground Contact Time

WPH ingestion was shown to have no effect on GCT during either the initial or repeated bout. The lack of any effect is was noted for other markers of muscle damage examined in the present study, but this may be as a result of the nature of the drop-jump task. Previous research has shown the detrimental performance effects of a bout of muscle damaging exercise to be masked by jumps that involve an active pre-stretch, therefore employing the stretch shortening cycle (Byrne & Eston, 2002b).

Byrne and Eston (2002b) suggest that one or more of the mechanisms proposed by Van Ingen Schenau et al. (1997) (the storage and release of elastic energy, available time for force production and potentiation of the contractile machinery) attenuates the detrimental performance effects of exercise-induced muscle damage. Ettema et al. (1990) supports Byrne and Eston (2002b) in suggesting that these mechanisms combine to enhance performance. It is possible that by enhancing the final muscle action, the pre-stretch somehow masks the decline in performance following muscle damaging exercise, in some way explaining the lack of difference between protein and control groups. If acute ingestion of WPH did not serve to enhance this mechanism it might explain the lack of treatment effect.

Sprint performance

There were no significant interactions across 5-10m and 0-20m distances, highlighting the absence of any effect of the acute administration of WPH. There was some however some evidence to suggest that WPH might facilitate the recovery of muscle function over a ten metre distance. There was some evidence of the RBE phenomenon noted by previous researchers, with a significant difference noted for bout across 0-10m.

The mechanisms responsible for reductions in sprint performance following muscle damaging exercise are unclear. Twist & Eston (2005) suggested that a reduced reflex sensitivity during the stretch-shortening cycle (SSC) may impair the ability to utilise ground impact forces, thus producing less force during the propulsive phase of the leg movement phase and increasing time in contact
with the ground. The increase in GCT noted in the present study would support this hypothesis. Previous studies (Chen et al., 2007; Dutto & Braun, 2004) have shown a reduction in force production, stride length and stride frequency in sub-maximal running following EIMD. If indeed these mechanisms are responsible for the decrements in sprint performance, the present study imparts some evidence that WPH may exert an influence on one or more of these. However, since it is still unclear whether these kinematic changes are evident during sprint running performance, further research is needed to investigate whether the ingestion of WPH has any effect on these mechanisms.

**Conclusion**

The acute administration of WPH following a repeated bout of damaging exercise appears to have no effect on the recovery of muscle function; however there is some evidence to suggest that WPH may facilitate the recovery of sprint performance at 48h.

These findings are in line with those of studies one and two in the present body of work showing that acute WPH ingestion has no effect on markers of EIMD, as well as those that support the presence of a RBE.
8. General discussion, conclusions and recommendations for further study

This body of work set out to examine the effects of WPH on the recovery of muscle function following a bout of eccentric exercise, whether a timing effect exists, and finally if the ingestion of WPH enhances the RBE.

All three studies showed that acute WPH ingestion conveyed no significant effect on the recovery of muscle function (GCT, squat jump), or ratings of muscle soreness following EIMD. There was limited evidence to suggest that WPH ingestion may convey some benefits to 5-10m sprint performance during the recovery from EIMD.

These findings are in line with the literature that shows protein consumption conveys no additional performance benefits when daily dietary protein needs are met (Phillips and Van Loon, 2011). One limitation of the present body of work is lack of dietary analysis to determine the subjects’ total energy and protein intakes. As stated previously, given the protein requirements to maintain performance and the average daily protein intake in the UK, it is unlikely that subjects’ diets were in an energy or protein deficit. However, confirmation of this would enhance the application of the findings of this body of work.

In explanation for the lack of effect noted; one possible hypothesis is the positive effect WPH has on the recovery of muscle glycogen (Morifuji, 2009). Morifuji (2009) suggested that if whey protein hydrolysate induces a strong insulinotropic effect it may result in enhanced performance or recovery via the stimulation of glycogen formation. Insulin may facilitate glucose entry into cells in greater amounts than needed for cellular respiration, leading to increased muscle glycogen concentrations prior to exercise and in the post-exercise recovery period. Each of studies in the present body of work used performance measures of muscle function to assess the impact of WPH. These were maximal in nature and of relatively short duration, therefore performance would not be affected by muscle glycogen concentrations. Additionally, the carbohydrate placebo used may in itself exert an insulinotropic effect, essentially mirroring the effects of the treatment group.
The inflammatory response is central to the model of muscle damage (Peake, Nasaka and Suzuki, 2011) with neutrophils and macrophages contributing to the degradation of damaged muscle tissue via the release of ROS (Cannon and St. Pierre, 1998). The inflammatory response is evident within several hours (Beaton et al., 2002) and remains for up to 14 days (Jones et al., 1986). The inflammatory response over this time period would not be influenced by acute WPH ingestion which may in part explain the lack of effects noted on the recovery of muscle function.

Finally, acute protein ingestion may only increase muscle protein synthesis for up to a few hours post-ingestion (Rasmussen et al., 2000). It is therefore unlikely to exert any influence over the recovery of muscle function during five days.

Given the various explanations in support of the lack of a treatment effect associated with the acute ingestion of WPH following EIMD, and the findings of this body of work, it is clear that there are no benefits to be achieved from ingesting WPH to facilitate the recovery from muscle damaging exercise.
References


muscle soreness, creatine kinase, and neutrophil count: a preliminary report. 


Appendix 1

Sample Risk Assessment

SECTION ONE
ADMINISTRATIVE DETAILS

REFERENCE: SSH/HAZ/0085
DEPARTMENT: Sport & Health Science
DATE: 30-11-2009
REMEDIAL ACTION REQUIRED? YES ☐ NO ☐
REMEDIAL ACTION PRIORITY? HIGH ☐ MEDIUM ☐ LOW ☐

WORK ACTIVITY: Protein ingestion prior to muscle damaging plyometric exercise
BRIEF DESCRIPTION: Healthy adult population will ingest whey protein supplement prior to/after muscle damaging plyometric exercise protocol. Measures of sprint performance and vertical jump taken

ESTIMATED No OF EMPLOYEES AT RISK: N/A
ESTIMATED No OF NON EMPLOYEES AT RISK: 40

SECTION TWO
HAZARD IDENTIFICATION

HAZARD = something with the potential to cause harm * Identify HAZARDS, circle KEYWORDS

HAZARDS

Physical
Confined space
Construction
Display Screen Equip't
Electricity
Environment
Fire
Handling
Heat / Cold
Housekeeping
Machinery
Movement
Pressure / Vacuum
Radiation (Ionising)
Radiation (Non Ionising)
Transport
Water
Weather
Chemical
Physical state
Properties
Routes of Entry
Biological
Type
Properties
Genetic modification
Psychological
Type
Other hazard(s): keywords: Protein Hydrolysate ingestion (commercially available product), muscular fatigue, joint impact, unstable technique

KEYWORDS

apnoeic, cold, hot, toxic, irritant, lone working, ventilation
CONCAM, Regs Ass't, scaffolding, work at height, falling object
DSE, Regs Ass't, desk chair, electricity, eye strain, eye test, posture
PAT testing, live static, induced arc, heat, burn, shock, 210V AC, 405V AC, high voltage
temperature, humidity, light, sound, space
flammable, combustible, explosion, oxygen, heat
abrasive, heavy lifting, pushing, pulling, sharp, hot, cold, awkward
radiation, conduction, convection, burn, scalds, touch
falling, tripping, slipping, storage, space, cables, combustion, sources, hygiene
MHO, Regs Ass't, cutting, rotating, sliding, falling, entrapment, breakage, ejection, of parts, electricity, radiation, heat, cold
slip, fall, trip, wet, ice, steps, stairs, height
burst, release, lines, joints, container, cylinder, explosion, leak, blockage, relief, control, failure
radioisotope, X-ray, alpha, beta, gamma, contamination, exposure, use, storage, disposal
ultra-violet, infrared, laser, microwave, burns, welding, eye, cataract
road markings, road signs, dangerous, loads, minibus, forklift, truck, trolley, truck, commercial, vehicle, passenger, lift, goods, lift, footpath, ramp, car, boat
diving, drowning, slipping, electricity
hot, cold, wet, ice, wind, lone-working, frostbite, heat-stroke, sunburn, skin cancer, hypothermia
solid, dust, liquid, gas, vapour, fume, hot cold
COSH H, Ass't, toxic, corrosive, irritant, carcinogen, allergen, flammable, unstable, explosive
inhalation, ingestion, skin contact
COSH H Ass't, microorganism, bacteria, virus, parasites, cell culture, storage, disposal
infectious, pathogenic, carcinogenic, mutagenic, teratogenic, storage, disposal
GMO, Regs Ass't, storage, disposal
fatigue, stress, trauma
SECTION THREE: RISK ASSESSMENT

RISK = a combination of the likelihood a hazard will cause injury and the severity of the injury

*Quantify risk for each hazard identified using the following table:

<table>
<thead>
<tr>
<th>Likelihood of injury</th>
<th>Score A</th>
<th>Severity of injury</th>
<th>Score B</th>
</tr>
</thead>
<tbody>
<tr>
<td>improbable</td>
<td>1</td>
<td>very minor injury</td>
<td>1</td>
</tr>
<tr>
<td>remote</td>
<td>2</td>
<td>minor injuries</td>
<td>2</td>
</tr>
<tr>
<td>possible</td>
<td>3</td>
<td>major injuries</td>
<td>3</td>
</tr>
<tr>
<td>probable</td>
<td>4</td>
<td>severe injury</td>
<td>4</td>
</tr>
<tr>
<td>likely</td>
<td>5</td>
<td>death</td>
<td>5</td>
</tr>
</tbody>
</table>

- Enter Hazards identified in Section 1
- Enter Existing control measures
- Quantify Risk factor by multiplying Score A and Score B, taking account of existing control measures.
- If Risk factor is over 5: take Remedial Action to improve Existing control measures or abandon the task
- If Risk factor is 5 or under, the risks are under adequate control, but should be carefully monitored

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Existing control measures</th>
<th>Score A</th>
<th>Score B</th>
<th>Risk (A x B)</th>
<th>Remedial Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey Protein Hydrolysate</td>
<td>The protein supplement to be used is commercially available and will be administered in small quantities (25g). All participants will be made aware of what whey protein does and that it will be administered within safe limits. Those with diagnosed with liver damage, malnutrition, defect of amino acid metabolism will be excluded from the study due to risk of amino acid homeostasis impairments.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Muscular Fatigue during drop jump and squat jump</td>
<td>Ensure participants are in full control during take-off and landing. Provide support where necessary. All participants will undertake familiarisation sessions</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Joint impact during drop jump and squat jump</td>
<td>Exclude those individuals with existing hip, knee, and ankle conditions</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Unstable technique during drop jump and squat jump</td>
<td>Ensure individuals are confident of completing exercise with good technique; provide support where necessary. All participants will undertake familiarisation sessions</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Trip or fall during sprint test</td>
<td>Surface checked for any foreign objects prior to commencement of activity. Correct footwear to be worn by participants. Participants will undertake familiarisation sessions.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 2
Participant health questionnaire

PHYSIOLOGY MEDICAL QUESTIONNAIRE

Name: ..................................................................................................................

Age: ........................................................

Are you in good health? Yes/No
If no, please explain:

How would you describe your present level of activity?
Vigorous: 
< once per month
once per month
2-3 times per week
4-5 times per week
> 5 times per week

Do you suffer, or have you ever suffered from:
Asthma Yes No
Diabetes Yes No
Bronchitis Yes No
Epilepsy Yes No
High blood pressure Yes No

Are you currently taking medication? Yes/No
If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? Yes/No

If yes, please give particulars:

Are you presently taking part in any other laboratory experiment? Yes/No

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experiment/exercise task if they:

- have a fever, suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- had any previous allergic or sensitivity response to dairy proteins;
- in the last three months you have undertaken resistance training of the quadriceps muscles;
- in the last 3 months have engaged in regular physical activity once per week or more involving repeated jumping and landing e.g. gymnastics, basketball, volleyball;
- in the last 3 months had you a knee, quadriceps or other musculoskeletal or medical problem which might interfere in your ability to perform the required exercise and tests;

DECLARATION

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. I undertake to obey the laboratory / study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw.

Signature of Subject .................................................................

Date: .............................................................
Appendix 3
Participant informed consent

I hereby voluntarily give consent to engage in an exercise science study. I understand that the test battery will involve tests during which I may be encouraged to work at maximum effort and that at any time I may terminate the test for any reason.

I understand there are certain changes which may occur during the testing process. They include abnormal blood pressure, fainting, disorders of heart beat, and very rare instances of heart attack. I understand that every effort will be made to minimise problems by preliminary examination and observation during testing.

I understand that I am responsible for monitoring my own condition throughout study, and should any unusual symptoms occur, I will cease my participation and inform the investigator of the symptoms. Unusual symptoms include, but are not limited to: chest discomfort, nausea, difficulty in breathing, and joint or muscle injury.

I understand that I will be required to consume a dietary supplement as requested by the investigator and that I am under no formal obligation to do so. If at any time I feel unable to consume said supplement I will inform the investigator and will be withdrawn from the test.

I understand that I am free to withdraw from the study at any point, without giving reason, and that all data recorded will be retained by Kristoph Thompson indefinitely in secure storage for the purposes of informing future research. I understand that upon completion of the study I will have the opportunity to be debriefed and receive a brief summary of results if I so wish.

Also, in consideration of being allowed to participate in the fitness tests, I agree to assume all risks of such fitness testing, and hereby release and hold harmless Kristoph Thompson, The University of Exeter and their agents and employees, from any and all health claims, suits, losses, or causes of action for damages, for injury or death, including claims for negligence, arising out of or related to my participation in the fitness assessments.

I have read the foregoing carefully and I understand its content. Any questions which may have occurred to me concerning this informed consent have been answered to my satisfaction.
Appendix 4

Information sheet for participants

The purpose of this study is to examine the effects (if any) of a widely available commercial nutritional supplement on the effects of exercise induced muscle damage and physical performance.

All subjects will be male, aged 18-23 and if chosen to participate in the study you will be randomly assigned to treatment or control groups. Familiarisation sessions will take place for all of the tests to be included in the study prior to its commencement (squat jump (SJ), time in contact with the ground following a drop jump (60cm), sprint performance (20m), acceleration (5-10m), and muscle soreness (Visual Analogue Scale - VAS)).

Baseline measures of each value being examined will be taken, followed by an exercise session involving vertical jumps. Nutritional supplement will be administered pre/post exercise depending on group.

You will be required to repeat the tests 24, 48, 72, and 168hrs after the exercise session. The tests should take approximately one hour to complete.

Certain participants will be selected to take part in another identical exercise session 21 days after the first. Identical tests will be administered 24, 48, 72, and 168hrs after the exercise session.

You are not eligible to take part in the study if during the previous 3 months; you have undertaken resistance training of the quadriceps muscles; have engaged in regular physical activity once per week or more involving repeated jumping and landing e.g. plyometrics, basketball, volleyball); had/have a knee, quadriceps or other musculoskeletal or medical problem which might interfere in your ability to perform the required exercise and tests; or had any previous allergic or sensitivity response to dairy proteins.

In addition, subjects will be required to satisfactorily complete a PARQ form and to give Informed Consent.

Upon completion of the study, all data shall be held in secure storage by the principal investigator (Kristoph Thompson) with controlled access for the purpose of informing future research. Additionally, participants will have the opportunity to be debriefed and obtain a summary of the study results.