The effect of a plant based protein source on the skeletal muscle metabolic and functional response to eccentric exercise

Submitted by Kiera Wilkinson,

to the University of Exeter as a thesis for the degree of

Doctor of Philosophy in Sport and Health Sciences

November 2023

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Abstract

Human skeletal muscle undergoes shortening and lengthening cycles of contractions as well as adaptive cycles of muscle protein turnover and remodelling. The role of dietary protein is to provide amino acids for the synthesis of new proteins, specifically skeletal muscle proteins, to ensure the health and functional ability of individuals across the lifespan. The principle aim of this thesis was to determine whether a plant-based protein supplement could accelerate recovery following a bout of damage inducing eccentric exercise and to determine if changes in muscle protein synthesis (MPS), over the initial 48 hours after exercise underpin any improvement observed.

Contrary to the prevailing narrative that leucine dose and subsequent plasma leucinemia variables serve as an independent determinant of post-exercise MPS responses, Chapter 3 challenges this notion, highlighting the complexity of factors influencing protein synthetic responses and that post-exercise postprandial MPS response cannot be predicted from any single plasma leucine variable.

Chapters 4-6 focused on the time course of recovery, encompassing both male and female participants, to assess the efficacy of a nutritional intervention providing ample protein and leucine following eccentric exercise. Chapter 4 demonstrates that a dose of ~20g of protein with ~2g of leucine, predominantly from animal derived protein isolates, supports recovery in recreationally active males. This accelerated recovery occurred by 24h and 48h post exercise suggesting that MPS responses earlier post eccentric exercise may have supported this accelerated rate of recovery. However, presented in chapter 5, a plant-based protein supplement (*Pisum Sativum*) with high leucine content and

no apparent amino acid deficiencies failed to support post eccentric exercise recovery.

Chapter 6, demonstrated that exogenous amino acid provision immediately following eccentric exercise tended to increase MPS and improve total isokinetic work 3h following exercise, suggesting a protective effect on subsequent damage inducing processes or that the exogenous provision provides sufficient substrate for muscle remodelling and the skeletal muscle adaptive response. However, this effect does not extend over 24 hours, as muscle functional recovery was not supported by the consumption of a plant based protein supplement and was not associated with elevated MPS rates over (Chapter 5). However, the rates of MPS over this time frame were notably high across both conditions, this was alluded to be due to an elevation in the endogenous provision of amino acids from muscle protein breakdown following damage inducing eccentric exercise. The sustained protein provision and exercise stimuli over 48 hours post-exercise led to elevated MPS rates and accelerated recovery, potentially when muscle protein breakdown had returned to basal levels.

This thesis underscores the intricate interplay of dietary protein sources, muscle protein synthesis, and exercise stimuli in shaping post-exercise recovery processes. The findings contribute valuable insights for future interventions seeking to understand the nuanced influence of muscle protein synthesis on muscle function after eccentric exercise.

Acknowledgments

I would like to thank, Professor Francis Stephens, for an incredible opportunity to work within the Nutritional Physiology research group. Your guidance and expertise has shaped my scientific abilities over my PhD journey. I am grateful for the guidance and expertise I have gained from Professor Ben Wall and Dr Jon Fulford.

Many thanks to the Nutritional Physiology Research group. The OG NPG: Tom, George, Alistair, Andy and Mariana thank you for all your support and guidance from when I started out and throughout the years. NPG next gen: Gráinne, Amy, Sam and Ino thank you for the coffee breaks, laughs and getting through it together. The NPG newcomers: Ellen, Freyja, Chris, Erika, Ros and Marianna it has been a pleasure to work with you.

I would also like to shout out a special group of work colleagues but most importantly great friends. Lunch club, Common Beaver and the Raddy trips have been a constant peak throughout and where I could share the many troughs over a coffee or a pint with a great group of lifelong friends.

G, our weekly runs and debriefs are a part of my PhD life that I will always cherish and always needed. Amy, who knew at the beginning of this journey I would be getting a best friend alongside a research partner. What a journey we have been on and coming out the other end after working together so intensely together shows we are suited to work together and be lifelong friends.

Thank you to all my friends who have stuck by me, been a distraction, a shoulder to lean on and most importantly smile and laugh with. Special thanks go to Rose for always listening and supporting me.

Dr Emma Wilkinson and Dr John Wilkinson, thank you for inspiring the scientist in me. Childhood memories of wrapping you up in bandages, has not turned into the Doctor we may have first expected, but a Doctor none the less. Hope you are proud of me.

Mason, thank you for always believing in me (especially when I didn't believe in myself) and supporting me through the highs and lows. Thank you for always bringing fun and adventure.

To my mum and dad, thank you for your unwavering support, love and dedication to everything I do. I can't thank you enough for always being my cheerleaders with every next chapter I go into.

Table of Contents

List of Figures

List of Tables

Declarations

All of the *in vivo* human data collection was carried out as part of a wider clinical trial, by myself and Amy Booth. Placebo group data within **chapters 5** and **6** have been included in a PhD thesis submitted to the University of Exeter by Amy Booth to address a separate research question. The data within **chapter 4** is a pooled data set from another clinical trial performed within the Nutritional Physiology research group. The PhD research was for this thesis was funded by Beachbody LLC.

Professor Francis Stephens, Professor Benjamin Wall, Dr Alistair Monteyne, Dr George Pavis and Dr Tom Jameson performed the skeletal muscle biopsy procedure used in **chapters 4-6**.

All of the analysis contained within this thesis was performed by myself, with the exception of, mass spectrometry analysis presented in **chapters 4-6**, was carried out by Doaa Abdelrahman at the University of Texas Medical Branch. Electron microscopy fixation and imaging was carried out by Dr Christian Hacker at the Bioimaging Centre, University of Exeter. Dr Jonathon Fulford performed the magnetic resonance imaging presented in **Chapter 5**, with the image analysis performed by myself.

I hereby declare that the present thesis has been composed by myself, and that it is a record of work performed by myself, except where assistance has been acknowledged. No part of this thesis has been submitted in any other application for a higher degree and all sources of information have been appropriately referenced.

Kiera Wilkinson 28th November 2023

Abbreviations

AA	Amino acid
ATP	Adenosine Triphosphate
BCAA	Branched chain amino acid
BMI	Body mass index
CK	Creatine kinase
ddH2O	Ultra distilled water
DOMS	Delayed onset muscle soreness
EAA	Essential amino acids
ECM	Extracellular matrix
EIF	Eukaryotic initiation factor
F - ACTIN	Filamentous actin
FBR	Fractional breakdown rate
FOX	Forkhead box
FSR	Fractional synthetic rate
GC-MS	Gas chromatography- mass spectrometry
IL	Interleukin
IL1RA	Interleukin 1 receptor agonist
LDH	Lactate dehydrogenase
MaFBx	Muscle atrophy F-box
MCP	Monocyte chemoattractant protein
MCSF	Macrophage colony stimulating factor
MHC	Myosin heavy chain
MPB	Muscle protein breakdown
MPE	Mole percent excess
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging
mTOR	Mechanistic target of rapamycin
MuRF	Muscle specific RING finger-1
NEAA	Non-essential amino acids
P70S6K	P70 S6 kinase
PKB	Protein kinase B
SEM	Standard error of mean
TNFα	Tumor necrosis factor alpha
TRAIL	Tumor-necrosis factor related apoptosis-inducing ligand

UPS	Ubiquitin protease system
VAS	Visual analogue scale

1 General Introduction

‘*If you have a garden and a library, you have everything you need.’*

Marcus Tullius Cicero

An ode to the COVID-19 pandemic lockdown in which the first year of my PhD was spent

1.1 Overview

Human skeletal muscle undergoes shortening and lengthening cycles as well as adaptive cycles of muscle protein turnover. The effective and efficient functioning of skeletal muscle is dependent on the complex cytoskeletal networks being in alignment and interacting efficiently. Skeletal muscle provides important mechanical and metabolic functions for the body. Mechanically, the main function of skeletal muscle is to convert chemical energy into mechanical energy to generate force, power and movement [1]. This impacts the ability of humans to undergo everyday activities and maintain health and independence. Metabolically skeletal muscle acts as a reservoir for amino acids, carbohydrates, and lipids, contributes to basal energy metabolism and consumption of oxygen and fuel during states of demand such as exercise [1].

Volitional dynamic movements (force generation and limb movement) of skeletal muscle are generated in the motor cortex of the brain, recruiting motor units and resulting in specific movement patterns, inducing mechanical, metabolic and neuronal perturbations [2]. This causes feedback mechanisms from the skeletal muscle which influences metabolic demands and induces an obligatory response to allow limited disruption to homeostasis [3]. Muscle responds mechanosensitively, meaning that changes that arise during the contraction of the muscle depend upon the magnitude of forces and stresses experienced [4]. This mechanical sensing from a single bout of exercise will trigger intracellular signalling pathways that regulate muscle protein turnover and allow the muscle to adapt to the exercise stimulus structurally and metabolically. This mechanical

loading, from a bout of exercise, is the most recognised regulator of skeletal muscle mass. However what happens when skeletal muscle is disrupted?

This thesis focuses on when this cytoskeletal network is potentially compromised following unaccustomed eccentric exercise and the consequential influence on muscle protein turnover and recovery, alongside how protein nutrition can influence this process.

1.2 Muscle contractions

1.2.1 Muscle structure

The basic structure of skeletal muscle is integral to understanding the functioning of the muscle and how it is affected by exercise, inactivity, disease and other lifestyle and health factors. This can be thought of as a hierarchal structure of contractile machinery that can be visualised macroscopically and microscopically to provide insight into the organised architecture of these proteins, which is presented in **Figure 1.1**. Skeletal muscle is connected to bones via tendons, which allows the movement of the body. This skeletal muscle is made up of many fascicles, fascicles are made of many muscle fibres (myofiber), muscle fibres are comprised of many myofibrils, these myofibrils are composed of many sarcomeres which are aligned in series to create the contractile unit of the muscle [5]. These sarcomeres are composed of actin (thin) and myosin (thick) filaments, identified with a striated pattern. This striated pattern (segmented at the A band) is intersected with the I band, H zone and Z -line, shown in **Figure 1.1**. The I band is the area including the Z-line consisting of thin actin filaments not overlapping with the thick myosin filaments. This area contains nebulin which provides structural stability for the actin filaments. Sarcomere units connect at the Z -line, α-actinin links across the Z-line and along the myofibril. This is where tension is generated during muscular contraction and force is transmitted from sarcomere to sarcomere, suggesting that the Z-line is directly involved in transmission of tension [6, 7]. The thin actin-containing filaments are anchored and extend toward the middle of the sarcomere, the M-line, where they interact with the thick myosin-containing filaments to form cross bridges, generate and transmit force and drive contraction [1]. This force is generated and transmitted longitudinally and laterally

within a fibre. Titin is an elastic protein that attaches to the Z -line and to myosin; it has been demonstrated to have a role in residual force enhancement after stretch [8] and is thought to more effectively transmit cross bridge forces to the Z-line [9] and be protective by resisting excessive strain allowing the sarcomere to return to resting lengths [10]. These contractile filaments are maintained in a highly ordered structure by these specialised proteins. This allows the rapid and synchronized generation of movement and force in specific directions [11]. Excitation-contraction coupling is the process whereby force is generated with the transmission of a nerve impulse (via the transverse tubule system) initiating the release of calcium from the sarcoplasmic reticulum. Calcium binds to the regulatory protein troponin C on the actin myofilament, initiates a cascade reaction whereby tropomyosin is blocked from the actin binding site which allows the interaction with the myosin head and cross bridges are formed, this allows the muscle to slide and form new crossbridges [12]. This cyclic interaction of myosin forming cross bridges with actin and the cytoskeletal structure, allows active force to be produced, and is termed the sliding filament theory [13]. Examples of the influence of these structural proteins are evident in muscular dystrophies or myopathies, with the absence or abnormal expression of these proteins highlighting their importance [11]. Exercise induced muscle damage is utilised to represent disruption which can occur to this architecture and affect the functioning of the muscle following strenuous and unaccustomed exercise.

A resistance exercise repetition combines both a shortening (concentric) and a lengthening (eccentric) phase. During a concentric muscle action, an increase in velocity causes a lower force to be generated, however when the magnitude of force exceeds that produced by the muscle, this causes the muscle to lengthen inducing negative work, this gives eccentric actions high force and low energy

cost properties [14, 15]. In this regard, during the eccentric component of resistance training the absolute load can be greater [16] and therefore isolating this lengthening movement allows for sufficient activation of motor units and muscle fibres. This thesis will focus on the lengthening (eccentric) phase of the movement and how we can use this model to induce muscle disruption.

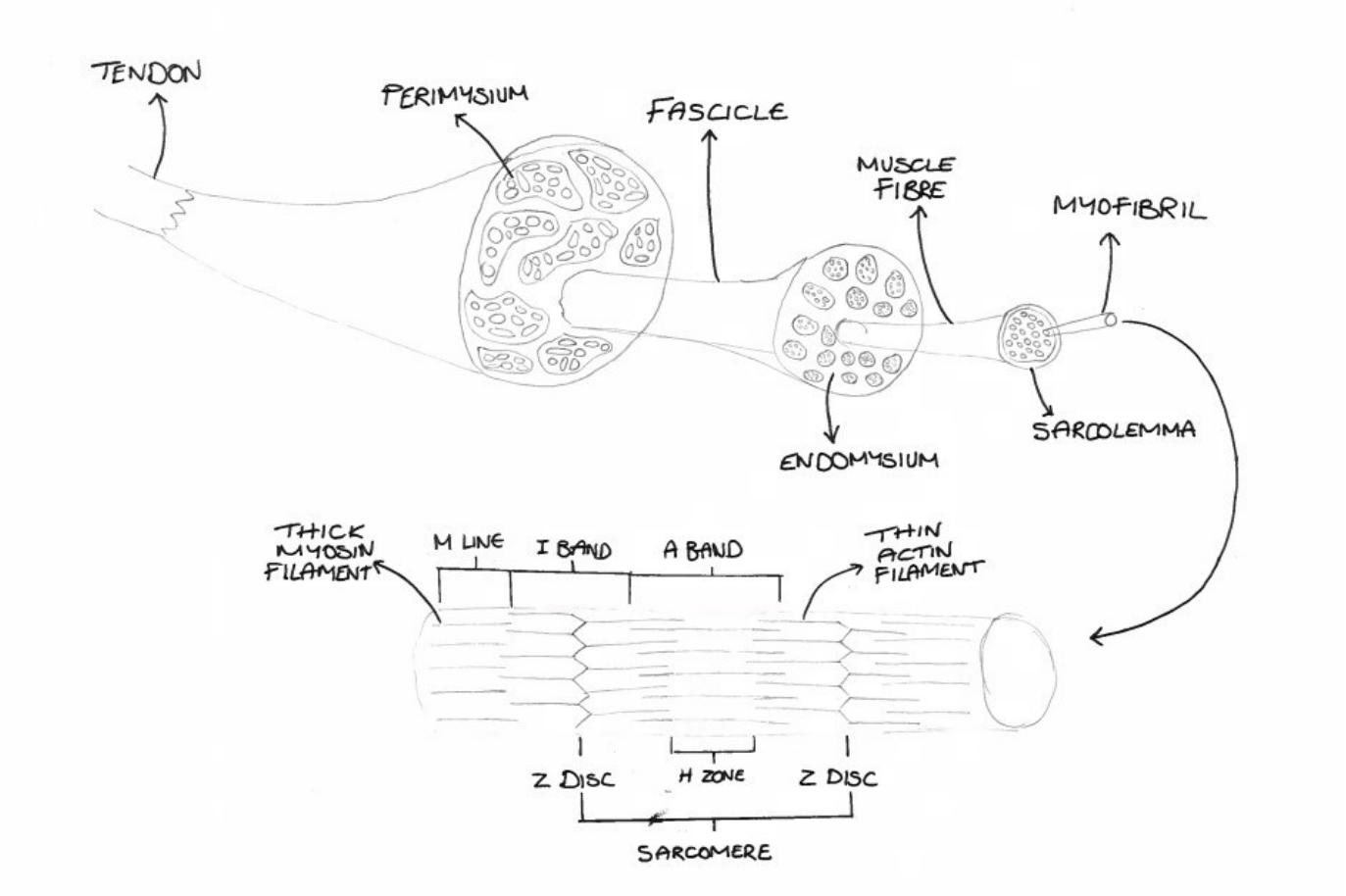

Figure 1.1 *Diagram to demonstrate the muscle structure, drawn early on in the PhD Journey to try and remember the key parts of the muscle structure*

1.2.2 Excentric exercise [17]

Eccentric exercise forcibly lengthens the contracting muscle [14], which creates greater tensile stress at lower metabolic costs than concentric contractions [15]. The descending limb of the length tension curve (**Figure 1.2**) is characterised by the muscle fibre being stretched during contraction to lengths longer than

optimum. When muscle fibres are stretched during a lengthening contraction, sarcomeres become stretched and muscle cross sectional area is reduced, this creates greater tension per active cross bridge and subsequently active muscle fibre [18]. The higher tension per active unit increases the tensile stress and the weaker sarcomeres in the myofibril absorb more of the stretch [19, 20]. Over repeated lengthening actions these sarcomeres reach a yield point where they are not able to withstand the tension and this will place stress on adjacent myofibrils [21]. When the muscle relaxes the myofilaments will not interdigitate properly causing further disruption to sarcomeres, this has been coined the 'popped sarcomere' hypothesis [22]. The cumulative effect of multiple contractions and the overstretching of sarcomeres will induce morphological changes, creating non uniformity of sarcomere length throughout a myofibre and damage to structural components. These may include disruption to the membranes of the sarcoplasmic reticulum, transverse tubules, the sarcolemma [19, 20] and titin scaffold [10], alongside structural changes to the myofibers.

Intense unaccustomed exercise induces changes to muscle homeostasis, these can be evidenced by direct and indirect markers of muscle damage. Various methods have been utilised experimentally. Commonly rodent models include crush, strain or freeze injury. Human models include downhill running, eccentric cycling, stepping, resistance exercise and isolated muscle actions of the knee extensors or elbow flexors either voluntarily activated or electrically stimulated. Voluntary activation involves the non-uniform activation of muscle fibres compared to electrical stimulation, whereby the recruitment pattern of muscle fibres is synchronous and uniform in the stimulated region. In animal models this consistently induces a substantial and rapid loss in force generating capacity,

marked infiltration of inflammatory cells and loss of cytoskeletal desmin [23, 24]. To produce these same findings in a human model it is more challenging and commonly is a lower limb model which induces increases in creatine kinase, losses in muscle strength and leads to the development of delayed onset of muscle soreness (DOMS). However there are a wide variety of protocols utilised to experimentally induce muscle damage which influence the magnitude of the stimulus and the potential for exercise induced muscle damage (EIMD). The severity of the disruption and therefore the time course of recovery will be influenced by the type, duration and intensity of exercise [25]. Understanding these mechanisms that induce muscle damage and that assist in recovery is relevant to allow continuation of daily living across a large proportion of the population**.**

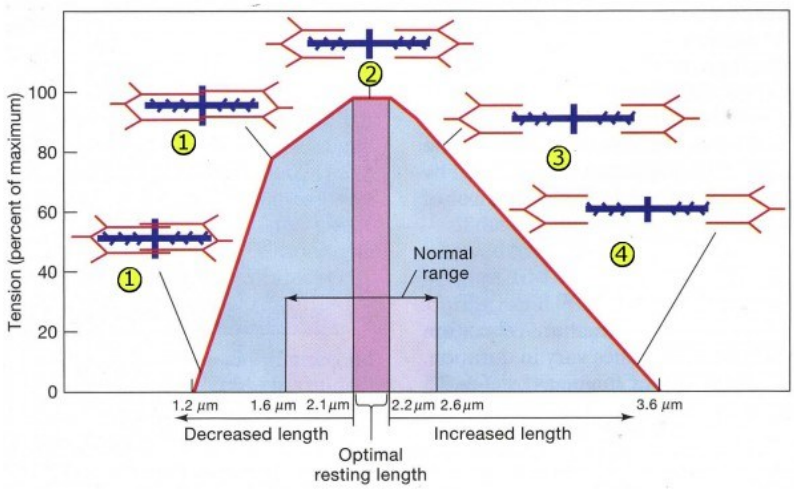

Figure 1.2 *The length tension curve demonstrating the extent of sarcomere changes with lengthening and shortening contractions*

1.2.3 Architectural responses

Morphological changes to the contractile machinery of the muscle, can be evidenced directly by assessing the muscle ultrastructure, utilising imaging techniques. Damage to the muscle has been termed myofibrillar disruption and

has been characterised as when the 'sarcomeres are distorted and out of register' [26]. It may extend across the I band or the entire sarcomere in a zigzag manner. Myofilaments terminate at the Z -line, this defines the lateral boundaries of the sarcomere [27]. These Z -lines, are the links between sarcomeres and act as an anchor for thin filaments; actin, titin and nebulin filaments. These structural characteristics suggest that Z -lines are more prone to mechanically induced injury due to the repeated stretching [28] and involvement with transmission of tension during a muscle contraction generated by the actin–myosin cross-bridge formation [6]. Heffner [26] denoted that in myopathic conditions the Z -line can often be seen streaming or smearing and is either no longer confined to the narrow zone within the I band or may extend across the I band or whole sarcomere. This Z -line streaming or smearing is suggested as a common event to accompany myofibrillar disruption.

Electron microscopy is used to investigate the level of ultrastructure disturbance to the myofibrillar and sarcomere structure. The area of disturbance originating from the myofibrillar Z -line showing a 'marked broadening, streaming and at places total disruption' are marked and the area occupied counted [28, 29]. Further from this, categories of myofibrillar disruption were determined. Focal areas of disruption were characterised as affecting one or two adjacent myofibrillar or sarcomeres. Progressing to a moderate area of disruption, characterised as 3-10 continuous sarcomeres and/or adjacent myofibrils [30-32]. Beaton, Tarnopolsky [33] added that moderate disruption would also include adjacent Z -lines showing obvious disruption. Extreme disruption has been characterised as if more than 10 continuous sarcomeres or adjacent myofibrils were counted in an area [30-32] and 10 or more continuous or adjacent Z -lines

demonstrated streaming [33]. Crameri, Aagaard [34] focused on Z -lines to look at ultrastructure changes after eccentric exercise and categorised this disruption as intact, disrupted or destroyed. Similarly, Yu, Carlsson [35] counted the number of fibres with irregular and amorphous changes to the Z -lines. Supernumerary sarcomeres were defined as ‘if one follows the Z-lines in transverse register across the myofibrils, one or more additional sarcomeres were present in some myofibrils. This characterisation has led to disruption being recognised immediately post eccentric exercise, evidenced with disrupted sarcomeres and Z -line streaming and the disruption developing further over 30h [30], 48h [32] and 96h [31] after exercise. The characteristics of Z -line disruption have been shown to be more pronounced following maximal electrical stimulation in humans [34].

Further indication of morphological change is the release of structurally bound contractile proteins such as myosin heavy chain (MHC), an increase in MHC is indicative of increased membrane permeability and degradation of contractile units [36]. Peaks in MHC have been evident 3 days [37] and 6-9 days [36] post eccentric exercise, with recovery of force generating capacity occurring 3 days post exercise [37]. Desmin intermediate filament, surrounds and links the Z -lines together. Animal models demonstrate an early loss of desmin following strenuous exercise [38], which has been suggested to be the reason as to why the myofibril becomes out of register. However recent work in humans does not support this, with increases in desmin and filamentous-actin (F-actin, stained with phalloidin) staining as an indication of increases in actin filaments and increase in the integrity of the Z -line in the days following eccentric exercise [39, 40]. Suggesting that the release of MHC, F -actin and desmin may be related to the focal regeneration and recovery of muscle function. It has been proposed that instead

of damage to the ultrastructure, the extent of disruption can be attributed to adaptive remodelling of the muscle [35]. With the focus of increases in muscle protein synthesis being orientated toward repair (sarcomeres in series) rather than hypertrophic changes (building sarcomeres in parallel) in the first 48hours post exercise [41] and subsequently allowing recovery of force generating capacity. This myofibril damage may actually reflect myofibril remodelling, which suggests that 'damage' is a functionally important response, and the repair allows the strengthening of the myofibre against further eccentric exercise [35]. Myofibrillar disruption has been evidenced to a greater extent with the lowering phase of an acute bout of strenuous exercise, in both trained and untrained individuals however the extent of the muscle damage was far greater in the untrained participants [32, 42], such that when a bout of strenuous exercise is performed again the decrements in performance and indirect markers of muscle damage will be less thus providing adaptive remodelling and a protective mechanism, termed the repeated bout effect [43].

1.2.4 Metabolic responses

Stretching, in the form of unaccustomed eccentric exercise, is likely to induce extracellular and intracellular membrane disruption, leading to a widened interstitial space [44], increasing the release of muscle specific proteins into the circulation [45] and the resultant release of chemokines. Alongside initiating stretch activated channels within the sarcolemma, increasing membrane permeability, promoting calcium influx and leakage of intracellular enzymes, causing the inability of the muscle to buffer changes in calcium, subsequently leading to excitation -contraction coupling dysfunction [46-48]. This decreases the degree of actin -myosin interaction and reduces the ability of the muscle to

produce tension, ultimately affecting the function of the muscle. A further impact of calcium influx, will lead to the activation of the calcium-activated neutral protease; calpain [47]. Calpains become focalised to the I -band and Z -line and other structural components, which will initiate degradation and disrupt the integrity of myofilaments [31, 47]. Blocking calpain activity (calcium channel blockers) has been demonstrated to effectively attenuate the contraction induced morphological disruption to the myofibre (Z -line streaming and desmin negative fibres) and reduce the rate of protein degradation following unaccustomed exercise [33].

Plasma concentrations of muscle proteins released into the systemic circulation have been utilised as indirect markers of muscle damage, such as creatine kinase (CK), lactate dehydrogenase (LDH) and myoglobin. Concentrations of these indirect markers of muscle damage, peak around 24-48 h post exercise. This is suggestive of increased membrane permeability, with a large variety of responses seen following exercise [49, 50]. However this increased release of markers of muscle damage do not correlate with reduced muscle function following eccentric exercise, suggesting no influence of markers on muscle fibre contractility [51].

The recruitment and infiltration of inflammatory cells occur to clear away debris in preparation for regeneration, tissue repair and remodelling of cytoskeletal matrix and skeletal muscle [52] and initiating the metabolic turnover of myofibrillar proteins. Leukocytosis involves the chemotaxis of macrophages and neutrophils to the injured muscle site, amplifying damage by producing lesions to muscle membranes and removal of necrotic debris [47, 53, 54]. Peaks in macrophages (CD68+) have been found at 24 h post eccentric exercise [34]. Macrophages

secrete pro-inflammatory cytokines such as tumour necrosis factor (TNFα), interleukin-1 and 6 (IL-1 and IL-6), locally into the muscle during and after exercise, which are then released into the systemic circulation [25]. Peaks in IL-6 have been evident 6 h post exercise coinciding with the greatest reductions in force generating capacity [37]. Pro-inflammatory macrophages (M1) have the phenotype plasticity to shift to an anti -inflammatory macrophage (M2). This shift is dependent on the tissue environment and is triggered by cellular debris [52, 53]. M2 (CD163) macrophages attenuate inflammation and promote muscle regeneration and growth by secreting anti-inflammatory cytokines (IL-10, insulin-like growth factor -1 and transforming growth factor -β1), stimulating myoblast proliferation and differentiation of the satellite cell pool [54]. The current understanding is that the early recovery phase is characterised by the overlapping processes of inflammation and occurrence of secondary damage. However, Lowe, Warren [55] concluded that the inflammatory process was focal and tightly controlled to the area of damage, and that muscle force was retained despite the processes of protein degradation and phagocytosis being initiated. The use of anti-inflammatory treatment, in the form of non-steroidal anti-inflammatory drugs (NSAID), has been shown to hinder recovery [56]. This therefore points to the idea that the inflammatory response is beneficial and involved in myofibrillar remodelling. It is still unknown whether these responses exacerbate the initial damage response or are due to the initiating event and are subsequently part of the repair process.

1.2.5 Functional responses

A failed muscle fibre according to Armstrong, Warren [18] is one that has ruptured or weakened muscle fibre structural components leading to a reduction in muscle

fibre performance, which can be characterised as the substantial loss of muscle function or strength. This indirect marker of muscle disruption can be measured as maximal isometric or isokinetic torque. The loss of muscle function appears immediately and may continue for days post unaccustomed eccentric exercise. Compared to work matched concentric contractions, high force eccentric contractions induce a greater degree of muscle damage, suggesting that force generating capacity loss is related to the high force element of the contraction [57]. The extent of myofibrillar disruption has been shown to correlate with loss of force generating capacity (r =0.89), with the greatest losses of muscle function amongst those with the greatest presence of myofibrillar disruption [31]. Similarly, 48 h after exercise, percentage decrease in maximal voluntary strength was significantly correlated to the extent of extreme myofibre disruption in untrained individuals after eccentric exercise of the *bicep brachii* [32]. With suggestions that if a 5% focal area of disruption is evident this should translate to a 5% reduction in the capacity of the muscle to produce force [58], however commonly this is far greater. Loss of strength following maximal eccentric exercise of greater than 25% [31, 33, 34, 49, 56, 59, 60] can be characterised as 'moderate exercise-induced muscle damage' and takes ~2-7days for muscle strength to recover [25]. To ensure that sufficient damage and recovery of muscle function could be investigated Ives, Bloom [61] withdrew participants from the data sample if they experienced a less than 10% reduction in peak isometric torque. Those experiencing a less than 10% reduction in muscle strength following a bout of high force eccentric exercise, may be protected from previous exposure to muscle damage [43, 62]. Eccentric exercise of the *bicep brachii* has been shown to induce greater losses in muscle function (>40%), compared to the knee extensors due to a greater novelty of the exercise stimulus in the upper

extremities compared to the lower extremities [63]. There also appears to be a maximal reduction in loss in force generating capacity, with it not exceeding 60% irrespective of the volume of exercise completed, indicating there must be a minimal level of muscle strength and function preserved for continuing exercise repetitions [64].

1.2.6 Soreness and swelling responses

This indirect measure of muscle damage relies on the individual to describe or rate the soreness experienced following exercise. Muscle soreness commonly arises when the exercise action is unaccustomed and high force; such that of eccentric actions [65, 66], generally the higher the velocity, tension and intensity produced the greater the soreness and subsequent damage [67]. Peak soreness arises 24-48 h following the high torque exercise bout [68]; arising the term delayed onset of muscle soreness (DOMS). The exact mechanisms associated with DOMS is still equivocal [21]; however there are plenty of suggestions as to what may cause this such as chemical, thermal or mechanical alternations [67]. The most common association is with peaks in circulating serum enzymes such as creatine kinase [68], released from leakage in the cell walls and disruption to the extracellular matrix (ECM), causing an acute inflammatory response and results in oxidative stress [69] which triggers nociceptors and manifests to be sensations of pain /soreness. Alongside degradation of protein components which are then released as protein bound ions, this exerts an osmotic pressure [70], creating stiffness and firmness experienced after eccentric actions and leading to oedema with the suggestion that this pressure activates nociceptors and elicits the feeling of soreness [71]. However there are very few studies confirming the increase in muscle volume associated with oedema [72]. Only

studies utilising measures of circumferences; with increases in upper arm circumference after eccentric exercise of the elbow flexors, however there were no correlations found between limb volume and soreness [73]. Overstretching of the muscle creates tension and mechanical strain across the connective tissue that forms a sheath around the myofibril bundles, therefore connective tissue damage is a suggestion for causing DOMS [74], with an increase in the urinary excretion of hydroxyproline, as an indicator of connective tissue breakdown, coinciding with the peaks in soreness [75]. With other suggestions of soreness localised to the musculotendinous junctions [76] induced by mechanical damage. The feelings of soreness and damage are often associated with myopathies however have also been experimentally induced in healthy young unaccustomed individuals. Soreness plays a role in limiting maximal strength; especially amongst those who haven't experienced it before, therefore alleviating these symptoms through nutritional intervention has been sought after. The short term suppression of feelings of soreness has been seen following the ingestion of branched chain amino acids (BCAA) [49], milk and carbohydrate [77] and whey protein blends [78]. Alleviating the symptoms over the short term is a benefit for those who need to perform repeated exercise sessions [79]. Whereas the effect of the repeated bout effect demonstrates a protective mechanism whereby the same stimulus in several weeks will not induce the same extent of DOMS [62, 80, 81]. It is important to understand the underlying mechanisms which cause damage to skeletal muscle and the mechanisms and processes behind the subsequent recovery from this damage.

1.3 Muscle protein turnover

Skeletal muscle mass is maintained by the parallel and opposing processes of muscle protein synthesis and breakdown. Relevant gains or losses of skeletal muscle mass are attributed to a persistent change in muscle protein synthesis rates (MPS), muscle protein breakdown rates (MPB), or a combination of both [82]. MPS and MPB will fluctuate diurnally, with repeated cycles of feeding and fasting [2]. The difference between these two processes represents the net protein balance. Amongst young, healthy individuals skeletal muscle turns over at ~1.2%·day^{-1}, allowing the remodelling of protein over time. During net muscle protein balance the rates of MPS and MPB are in equilibrium. There are many factors which can alter these processes, which consequently leads to acute or chronic perturbations in muscle protein balance. If this balance is shifted, a small decrease in synthesis and an increase in degradation can lead to the loss of muscle mass. An example of this imbalance is during periods of muscle disuse and unloading such as bed rest, limb immobilisation and other forms of inactivity [83, 84]. The imbalance with either a decline in MPS, increase in MPB or a combination of both, leads to skeletal muscle atrophy [85]. Similarly aging is characterised by a progressive loss in skeletal muscle mass, which causes reductions in muscle strength and function, commonly termed sarcopenia. This involves an imbalance in muscle protein turnover; increased muscle proteolysis, reduced MPS [86] and an insensitivity to anabolic stimuli including amino acids [82].

Two of the most potent anabolic stimuli to skeletal muscle are resistance exercise and dietary protein ingestion, and together they facilitate muscle protein accretion. Resistance exercise increases rates of MPS and MPB [87, 88],

however without the provision of exogenous amino acids net muscle protein balance will remain negative [87-89]. Bohé, Low [90] provided suggestions that the extracellular provision of amino acids stimulates MPS. This has led to an abundance of studies indicating that essential amino acids (EAA) are the drivers of MPS [91, 92]. With the infusion [93] or ingestion of free amino acids (AA) [94] or a combination of AA and carbohydrate [95], with as little as 6g EAA [96], after exercise having the ability to stimulate maximal anabolism.

Dynamic changes in muscle protein turnover and the early activation of signalling proteins, encompass acute responses to exercise, which may act as surrogates of long-term phenotypic changes in muscle mass and strength [3]. Muscle hypertrophy is obtained when muscle protein balance is positive, whereby rates of MPS exceeds that of MPB and new satellite cells are accrued in pre-existing muscle fibres. This is largely determined by the ability to increase protein translation initiation, through the activation of the mechanistic target of rapamycin (mTOR) protein kinase, which exists as two multi -protein complexes (mTORC1 and mTORC2). mTORC1 potentiates the phosphorylation of initiation factors; eukaryotic translation initiation factor 4E (EIF4E) and p70 ribosomal protein S6 kinase beta-1 (p70S6K1), for cap dependent translation initiation and elongation, and regulates translation of ribosomal proteins, concomitantly leading to a rapid rise in MPS [91, 97]. Gene expression of these pathways, such as increases in expression of EIF4E in exercised muscle [98], demonstrates a translational capacity that enables greater MPS rates. The importance of this and many other cellular pathways and networks can be identified when key pathways are absent or blocked [99]. Activation of the mTORC1 signalling pathway is known to respond to insulin [91], amino acids (specifically leucine [100]) and muscular

contraction [101]. Whereby the inhibition of the mTORC1 pathway by rapamycin abolishes the influence of exercise contraction and amino acids on MPS rates [102]. More specifically, the essential amino acid, leucine, is thought of as the key anabolic signal for MPS [103]. To understand the extent of the influence of leucine on MPS, studies have been conducted whereby leucine alone [104] and leucine added to inadequate protein dose [105] has maximised the MPS response. Leucine is sensed by sestrin2 [106], providing a signal for the stimulation of MPS, through activating the mTOR pathway [91, 107] and a signalling cascade that activates translation initiation. However measures of pathway phosphorylation are a snapshot of time, taken at specific time points post exercise and may form a disjunction between measured protein turnover events and the enzymatic expression of certain pathways.

1.3.1 Measurement techniques

The use of stable isotope tracers has allowed *in vivo* metabolic research into skeletal muscle protein metabolism to advance. A stable isotope is a chemically and functionally identical molecule to a naturally occurring element but differs in mass due to additional neutrons in the nucleus [2]. Common non -radioactive stable isotopes used in metabolic research include hydrogen (^{2}H), carbon (^{13}C) and nitrogen (^{15}N). These isotopes can be incorporated into organic molecules. The incorporation of labelled amino acids, such as [^{13}C] leucine and [^{15}N] or [^{2}H] phenylalanine, into the precursor pool (systemic circulation via blood sampling) is reflective of the subsequent synthesis of new proteins, with the assumption that the precursor pool stays the same throughout the sampling period [108] and there is a uniform distribution of amino acids throughout the pool [109]. The use of a primed continuous infusion attempts to achieve equilibration of labelled amino

acids in the precursor pool. This method requires the intravenous infusion of labelled amino acids, within a controlled laboratory setting, with a flooding dose or primed continuous infusion. The purpose of providing a priming dose of tracer before commencing a continuous infusion is to instantaneously label the total miscible pool of the metabolite of interest to the equilibrium enrichment that will be reached by the continuous infusion [110]. However a limitation of this method is that it does not allow the ability to measure muscle protein synthesis rates over extended periods of time (>12hours) as it is confined to within a controlled laboratory environment.

The use of deuterated water (^2H_2O) overcomes this limitation. Deuterated water equilibrates within the body water pool, due to the ability to diffuse through all body compartments, so once orally administered, will rapidly mix through the body within ~6hrs [111, 112]. Due to this dispersion throughout the body water pool, dosing protocols within this thesis, are based off individual body mass due to ~50-70% of body mass being body water [113]. The deuterated water equilibrates in the body water pool (intracellular precursor pool) and through the exchange of hydrogen (^2H labelling of carbon bound hydrogens of amino acids) through transamination reactions, the deuterium will be incorporated intracellularly to metabolic pools and tissues (myofibrillar protein pool). All amino acids can become ^2H labelled at their carbon position, however the non-essential amino acid, alanine has the potential for 4 ^2H labelled sites and is therefore easier to detect using isotope ratio mass spectrometry (IRMS). The total ^2H hydrogen labelling of alanine is ~3.7 times that of ^2H-labelled body water [111, 114]. This difference in ^2H labelling of the body water (precursor) and myofibrillar proteins (product) is taken into consideration when calculating myofibrillar protein synthesis using the precursor- product equation. The turnover rate of body water

is ~9%.d^{-1} [115], therefore maintenance doses of deuterated water are provided to maintain ^{2}H body water enrichment over the cumulative time frame for the investigation of MPS. This maintenance of the body water enrichment is beneficial for physiological research, as individuals can be safely investigated whilst continuing with their habitual or research restricted lifestyle (i.e. controlled diet and exercise) to allow the quantification and capture the variation in the protein synthetic response over days to weeks [41], whilst capturing the influence of repeated exercise, meal ingestion and different postprandial and post-absorptive states. Thus allowing a more integrative measurement of MPS than a tracer infusion. The use of the deuterated water method in humans has captured temporal patterns of myofibrillar MPS over 7 days [116], 8 days [115] and 6 weeks [117] of resistance exercise training.

The continuous infusion or the loading and top up doses of a tracer increases plasma enrichment, this tracer is then taken up by the muscle into the intracellular pool, which subsequently becomes incorporated into skeletal muscle protein. With the use of serial skeletal muscle biopsies the change in isotopic enrichment of skeletal muscle protein reflects the rate of muscle protein synthesis (corrected for natural abundance). The fraction of the protein pool that is new over a period of time is expressed as fractional synthetic rate (FSR) and is expressed as %·h^{-1} or %·d^{-1}. The fraction of the protein pool that is new can be quantified as myofibrillar (contractile protein only) or mixed muscle proteins which includes muscle protein subfractions; mitochondrial, sarcoplasmic, collagen. **Chapters 4, 5** and **6**, utilised a deuterated water ($^{2}H_2O$) dosing protocol, to allow the participants to continue with 'free living' conditions and allow the measurement of cumulative myofibrillar protein synthesis over extended time frames.

1.3.2 Muscle protein breakdown

Skeletal muscle protein is the body's primary labile endogenous source of amino acids [118]. The measures mentioned above do not consider the breakdown of muscle protein and therefore do not provide insight into the regulation of muscle protein turnover. However the measurement of muscle protein breakdown has been more challenging. The breakdown of proteins allow adaptation to new physiological conditions, provides amino acids for gluconeogenesis and the synthesis of new proteins. Severe burn patients are an example of an extreme catabolic state; this stress on the body imposes a greater demand for amino acids from muscle protein breakdown and replacing these amino acids is vitally important for survival [119]. *In vitro* and *in vivo* measurements show that denervated and unweighted muscles, demonstrate a loss of muscle protein which is as consequent of decreased protein synthesis and accelerated protein degradation.

Overall rates of protein degradation can be measured most readily through the accumulation of an amino acid that is neither synthesised nor metabolised in tissue. The appearance of 3-methylhistidine in the urine has been used as an indirect marker of proteolysis [120, 121]. N -methylhistidine, is released from myofibrillar proteins actin and myosin when these proteins undergo proteolysis [122]. Due to it not being able to be reincorporated this reflects the breakdown of myofibrillar proteins. Other methods to investigate changes in MPB include mRNA expression of proteins that compromise the molecular pathways of MPB, suggesting that MPB is regulated through multiple cellular signalling cascades. These are distinct systems that regulate protein catabolism; the ubiquitin-proteasome pathway (UPS)[123], lysosomal autophagy [124], calpain Ca^{2+}

dependent degradation [125] and caspase mediated degradation [126]. UPS, is an adenosine triphosphate (ATP) dependent pathway that is the main proteolytic system responsible for the degradation of normal and abnormal proteins [127]. This system plays a key role in accelerating the breakdown of myofibrillar proteins. These degradation pathways are controlled by the transcription factor forkhead box 03a (Fox03a), this is responsible for increasing the expression of E3 ubiquitin ligases: muscle specific RING finger-1 (MuRF1) and muscle atrophy F-box (MaFbx). These have bindings sites on the M -line and have been found focalised to the Z -line with the function of titin based degradation [128] and has been clearly linked to regulating protein degradation [124, 129-131]. Following 5 days of lower limb immobilisation, gene expression for myostatin, MAFbx and MURF1 were upregulated, suggestive of an increase in UPS mediated MPB leading to atrophy of skeletal muscle [132] and declines in muscle strength [84].

MPB and proteolytic gene expression have been shown to be elevated post resistance exercise; with peaks in MuRF expression seen at 1h [130], 6h post exercise [129] and returned to baseline by 24h [129], but MaFbx expression remaining the same over these time frames [129, 130]. Following eccentric exercise MuRF expression has been elevated [133], this was reduced with the provision of AA following exercise. These pathways and signalling cascades have been shown to be inhibited by Akt and PKB, this can be activated by circulating insulin [134] and from carbohydrate feeding following exercise to reduce MPB [135]. This aligns with the suggestions that the provision of protein post exercise assists in the remodelling and attenuates the breakdown of muscle, specifically be reducing the expression of muscle protein breakdown related genes.

Cytosolic microtubule-associated protein 1 and 2 light-chain 3 (LC3B-I, -II) is associated with lysosomal autophagosome formation and degradation [124, 130]. LCB11 expression was not altered by resistance exercise however was dampened by protein and carbohydrate feeding following exercise [130], suggesting the potent inhibition of autophagy pathways with nutrition and the role of insulin in depressing MPB pathways and increasing MPS via increasing Akt and mTOR phosphorylation. This suggests that modest amounts of insulin from carbohydrate feeding could have the potential to limit protein breakdown post eccentric exercise, however there is scope to measure this in relation to muscle protein turnover to understand if myofibrillar proteins are affected.

The methods mentioned so far to assess muscle protein breakdown are indirect markers and do not quantify specific muscle protein breakdown rates. Total protein breakdown is the sum of the rate of appearance of AAs into plasma, however the release of AA from protein breakdown can be directly reincorporated into protein without appearing in plasma [136]. The arterial-venous balance method was used to demonstrate increases in muscle protein breakdown following eccentric exercise [137], with phenylalanine kinetics across the artery, vein and muscle indicating that amino acids released from muscle protein breakdown can be incorporated into the muscle. Phenylalanine has been utilised as a tracer to determine fractional breakdown rates (FBR) due to the tracer not being oxidised or synthesised by the body, therefore the net appearance of phenylalanine signifies rates of muscle protein breakdown. This tracee release method has been utilised by Zhang, Chinkes [138], Phillips, Tipton [88] and most recently Pavis, Abdelrahman [139], whereby the intracellular dilution of amino acid enrichment from both arterialised plasma and the muscle bound pool after

the cessation of the continuous infusion is measured and used to calculated FBR. Following resistance exercise rates of mixed muscle FBR were increased 31% above resting at 3h and remained elevated (18%) 24h after exercise, however at 48h FBR had returned to resting values. These values were measured alongside FSR measurements which allowed the assessment of muscle protein balance, with protein balance remaining negative following exercise, which is expected as the study was undertaken in a fasted state.

Current evidence of MPB does not consider compromised muscle, due to the myofibrillar disruption that is evident following eccentric exercise [42], one might expect there to be a greater rate of FBR, due to the structural changes to the contractile machinery of the muscle. To date the only study quantifying rates of protein breakdown post eccentric exercise was studied in mice utilising a tyrosine release method [140]. Lowe, Warren [55] found reductions in muscle function, alongside increases in phagocytic infiltration which coincided with increased protein degradation rates. Degradation rates were rising by 24h post injury and plateaued at 60% above baseline from 48h onwards. However when protein degradation rates increased and phagocytic cells invaded this did not lead to a greater reduction in muscle function, suggesting that these are focal areas of damage and that degradation will play a role in adaptive regeneration and remodelling following damaging exercise [55]. The current view and consensus on MPB is that following exercise, without the provision of protein that muscle protein balance will remain negative. With the addition of protein, this assists in the inhibition of MPB, rather than a stimulation in MPS, to create a positive protein balance [141]. By providing protein nutrition does this spare skeletal muscle?

In a perioperative state the infusion of AA restored net muscle protein balance with MPS increasing, meaning that muscle protein is no longer needed as the precursor for MPS [118]. Is this the same following eccentric exercise? This pathway is underexplored and has potential implications for post exercise recovery.

1.4 Factors influencing muscle protein turnover

1.4.1 Exercise and muscle protein turnover

Resistance exercise, whereby the muscle resists an external load, has been shown to increase MPS, since early reports by Chesley, MacDougall [89] utilising a stable isotope tracer methodology and performing a unilateral exercise model to measure rates of mixed MPS. The intensity [142], volume [101], time under tension [143], contraction mode [144] and inter set rest intervals [145] at which the exercise is performed have all been shown as variables that can dictate the anabolic response. The optimal being considered a maximised response in MPS which will be determined by the mechanical sensing of the muscle to gauge these stimuli, this alters the metabolic and hormonal lieu and determines how the signal will be transformed that will lead to intramuscular increases in protein synthesis [146]. This provides the thesis that muscle activations purpose is to activate these intramuscular signalling pathways to increase MPS and subsequently over time accrue skeletal muscle proteins and induce muscle hypertrophy. Rates of mixed muscle protein synthesis, increase within 3 h after a bout of resistance exercise [137] and has been demonstrated to remain elevated for up to 24 h in trained [89, 147] and up to 48 h in untrained individuals [88]. An untrained individual is classified as not engaging in any specific structured exercise training programme/

regimen and can be viewed as naïve to resistance training [148]. Compared to a trained individual who has engaged with a strenuous and progressive resistance training programme for a period of at least two years (training at least 3 times per week) [149]. These differences in responses between trained and untrained muscle denote that when the resistance exercise stimulus is novel, this induces greater disturbance to the homeostatic balance of all muscle subfractions [30], with mixed muscle protein synthesis increasing by 132% [150] and 112% [151] compared to trained muscle after an exercise bout. In a trained state the stimulus required to produce a maximised MPS response is lower, but the response is rapid which may be due to an improved translational efficiency and potentially due to a higher resting mixed muscle MPS but the response is less long lived [151-153]. With training, the perturbations in muscle homeostasis will be reduced, when muscle damage progressively subsides [154] and the MPS response can be directed towards supporting muscle hypertrophy (building sarcomeres in parallel), rather than supporting repair and recovery [35, 41]. Resistance exercise training attenuates the acute mixed muscle MPS response, however this attenuation has not been evident in myofibrillar protein fraction [151, 152] which may be due to the demand for myofibrillar protein synthesis over a training period to accrue new myofibrillar contractile proteins [41, 152]. Due to the structures of muscle that produce and emit force during exercise contractions being proteins, it is commonly thought that there is a relationship between myofibrillar disruption and protein metabolism. The focus of this section so far has been on resistance exercise contractions which utilise both concentric and eccentric movement phases. However when movement phases are isolated MPS responses differ. Earlier work comparing contraction mode did not see differences between lengthening and shortening contractions ability to stimulate MPS, however

Moore, Phillips [144] found differences between the modalities in myofibrillar protein synthesis over 4.5 h. These differences may have been apparent due to work matching the contraction modes therefore the same relative intensity of exercise was performed, whereas earlier studies performed the eccentric work at the work rate of 80% of concentric 1RM, which would not produce as much voluntary eccentric force. Eccentric exercise has been shown to have a larger post exercise MPS stimulus, however this comes hand in hand with a potential disruption to the muscle ultrastructure and ensuing inflammatory degradation which requires the repair and replacement to occur, thus a potentially greater rate of MPB, alongside an upregulation of MPS, which alludes to the idea that exogenous provision of amino acids could aid this process, spare muscle proteins from being broken down and assist in recovery from eccentric exercise and improve the muscle protein balance.

1.4.2 Nutrition and muscle protein turnover

The effect of feeding on MPS has been extensively investigated in humans; whereby the effect of amino acids or a source of protein have robustly increased MPS over several hours following consumption [93, 155]. Amino acid (AA) availability in the extracellular pool will stimulate MPS and activate the movement of AA into the intracellular pool [155], this intracellular pool needs to have an availability of precursors for *de novo* MPS. The postprandial increase in plasma AA concentrations stimulate whole body protein synthesis but also has the potential to inhibit proteolysis [87], which results in a positive whole body protein balance [95, 156]. An increase in the concentration of plasma amino acids, determined by the digestion and absorption kinetics of the protein source, can have a measurable effect on muscle protein synthesis, with a latent period of 30

minutes after commencing a constant intravenous infusion of AA, MPS increases between 30-120minutes postprandial [157]. Similarly following a single dose of orally administered protein (48g whey protein, 20g EAA) a latent period of 45 minutes has been demonstrated before MPS rates tripled between 45-90minutes [158]. The optimal composition of a protein source has been extensively investigated to support maximal muscle protein anabolism. Specifically the digestion and absorption kinetics of a protein source and subsequent degree of aminoacidemia are thought to play a key role; with the consumption of high-quality and rapidly digested proteins stimulating the most robust increase in rates of MPS in healthy young adults [159-161], these concepts will be further explored in *Section 1.5*. The provision of 6g of EAA [96] and 6g EAA plus 35g CHO [95] has been shown to be equally effective at stimulating MPS. Insulin secreted in response to nutritional stimuli appears to play a permissive role, with low rises in insulin required to facilitate optimal MPS rates [134]. Similarly, 10g rapidly digested free form EAAs, equating to 20g of intact protein, has maximised the MPS response in rested untrained muscle [162]. The exogenous provision of EAA are necessary for the stimulation of net muscle protein synthesis such that non-essential amino acids (NEAA) have no additional benefit to the acute MPS response [92]. It has been hypothesised that leucine as a single nutrient can elicit a similar signalling response as would a complete amino acid mixture and that the removal of leucine from an amino acid mixture would decrease this stimulatory response [163]. Both leucine alone [104] and leucine added to inadequate protein dose [105] have been found to maximise the MPS response, such that extracellular leucine concentrations determine the activation of synthetic processes. Once synthesis is activated the inward transport of amino acids increases to serve as precursors and sustain MPS over later postprandial

periods [155, 164], therefore the requirement for a complete amino acid mixture rather than leucine alone becomes apparent. The influence of leucine on anabolic responses has been investigated predominantly to improve the sensitivity of anabolically resistant aging muscle to protein nutrition such that higher levels of leucine have been demonstrated to increase acute MPS responses [165-167] and as such reach the threshold to maximise the anabolic response. When considering a protein source, rather than constituent amino acids, 20g protein providing 2-3g leucine, is considered the optimal dose to stimulate rates of MPS which are maximal [168, 169], above this level a rise in amino acid oxidation is prevalent. Suggesting that the muscle is saturated or that beyond a certain threshold there is a mechanism by which the muscle becomes desensitised to the effects of amino acids [158]. The ~20g high quality protein ingested is adequate to overcome the stimulatory threshold, required to increase translation initiation and provide sufficient building blocks in the form of amino acids to form new polypeptide chains.

1.4.3 Exercise plus nutrition

In the absence of amino acids immediately post -exercise, the net balance of muscle proteins remains negative until nutrient intake occurs. The primary source of amino acids during this period is derived from endogenous sources, primarily resulting from an elevated rate of muscle protein breakdown [88, 137]. Feeding can suppress the increase in muscle protein breakdown, counterbalancing the negative protein balance following resistance exercise [93, 94, 155]. Resistance exercise and postprandial hyper-aminoacidemia both independently stimulate MPS. The synergistic effects of these stimuli result in an amplified MPS response, facilitated by the delivery and inward transport of amino acids to the muscle [87].

Resistance exercise in a postabsorptive state can accelerate MPS by ~100%, while under hyperaminoacidemia this can enhance MPS by more than 200% [87, 93]. Following exercise in the fed state (25g whey protein), myofibrillar protein synthesis has a rapid elevation over 1h and is significantly elevated over the early (0-2h, 179%) and later (204%) postprandial periods compared to fed only (103%)[170], such that the feeding post exercise determines the amplitude and duration of protein synthesis. In support of these findings, greater rates of MPS are also evidenced alongside greater mTOR phosphorylation following exercise and feeding, implicating amino acids as pivotal stimuli for signalling pathways to upregulate MPS [171]. Sustaining a high protein intake in conjunction with regular resistance exercise results in a positive protein balance, fostering the accrual of muscle protein and an increase in fat free body mass over an extended period [172].

A multitude of studies support the notion that exercise sensitises the muscle to protein ingestion [137], with pronounced increases over 0-6 h post resistance exercise. The use of a unilateral exercise model allows the attribution of changes in MPS to the respective feeding experienced by the exercised leg compared to a contralateral control leg. These studies therefore can refer to the extent of differences between the exercised and non-exercised legs and clearly demonstrate that the nutritional supplement combined with exercise elicits a greater anabolic response than nutrition alone. Nutritional strategies that support MPS as the primary driver of positive changes in muscle protein net balance encompass a variety of protein sources; milk [173], whey [160, 174], casein [175], egg [176, 177], beef [178] and the emergence of plant based protein sources as

a means to maximise the anabolic response of exercise [179, 180] and result in a positive protein balance [181, 182].

These studies have looked at characterising the response of exercise and nutrition over an acute time frame of 0-6 h postprandial post -exercise however it is worth noting that the stimulation of MPS is thought to be amplified for at least 24-48h post exercise and is characterised by enhanced sensitivity to amino acids [183]. Maximal fibre activation (90% 1RM to failure and 30%RM failure) sensitised muscle to feeding of whey protein (15g) 24hours into the post exercise recovery period, with the exercise potentiating the feeding induced increase in rates of myofibrillar protein synthesis [183]. This response to exercise stimuli and feeding indicates that repeated bouts of exercise over subsequent days may assist in maintaining an increased rate of MPS and subsequent improvement in functional exercise recovery. However this process would be dynamic over days of recovery therefore requires the utilisation of deuterated water loading protocols to allow the measurement of cumulative changes in MPS over several days, rather than the snapshot provided from acute tracer studies. Gasier, Fluckey [114] demonstrated that over an acute (but extended) 16h recovery period MPS was greater in the exercised leg than the control leg. Similarly Wilkinson, Franchi [115] and Holwerda [116] demonstrated that a resistance exercise stimulus daily (over 3 days) provides a large (~1.79%·d^{-1}) anabolic increase compared to the contralateral rested leg. This provides the rationale to the study design within **Chapter 4** and **5**, whereby concentric exercise is performed daily to maximise the MPS response to assist in recovery alongside the addition of a protein nutritional intervention.

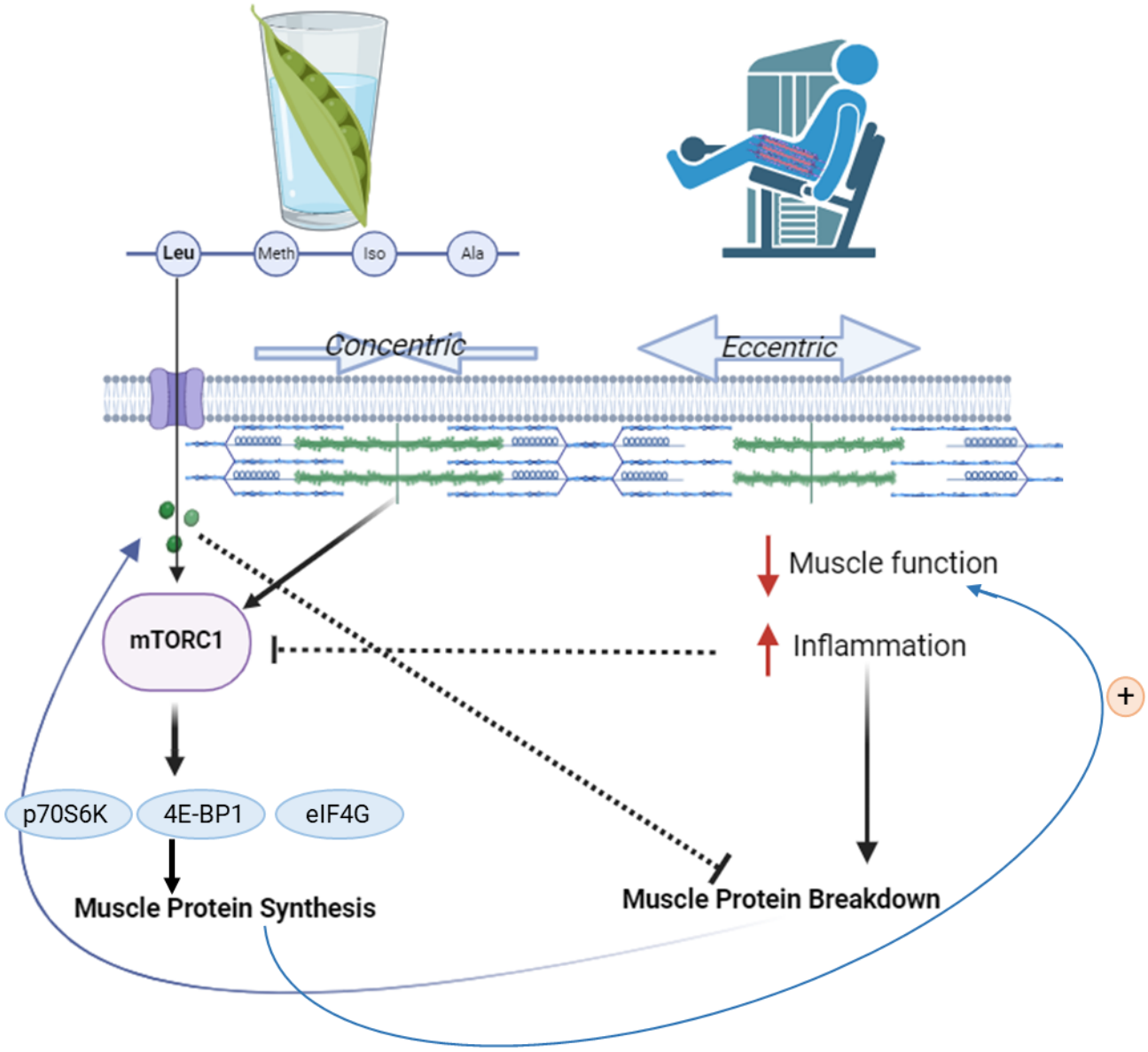

Figure 1.3 *Summary of key pathways associated with muscle contraction and protein nutrition. Dashed line indicates inhibition of a pathway. Solid line indicates stimulation of a pathway.*

1.4.4 Protein nutrition and eccentric exercise recovery

Eccentric exercise induces a transient decline in force generating capacity and this is attributed to mechanical damage to the contractile proteins of the muscle fibres. This subsequent response is tightly controlled with the purpose of these regulatory processes (**Figure 1.3**) to restore muscle function. The ability of the

muscle to recover from eccentric exercise is importance for the preservation and unimpeded execution of daily activities. This is particularly crucial for individuals engaged in recurrent exercise sessions or those seeking to avoid exacerbating exercise-induced damage while maintaining their routine activities. The exploration of precise interventions capable of facilitating the restoration of muscle function and alleviating the symptoms associated with muscle damage is highly sought after.

Protein supplementation has been widely used as a intervention to accelerate recovery following intense exercise [184]. The ingestion of whey protein hydrolysate (25g and 31g) has been shown to improve recovery of muscle force generating capacity, within 6 h and 24 h following 100 eccentric contractions of the knee extensors [59, 61]. Similarly strength (peak torque) and function (total isokinetic work) recovery from 60 eccentric contractions of the knee flexors was accelerated with milk (34g) and milk-based protein and carbohydrate (33.4g) supplementation [77]. Following 300 eccentric contractions of the knee extensors a whey protein and casein blend pulsed (4 x 20g over 9h) over the acute hours post exercise and a single dose every day thereafter, accelerated the rate of skeletal muscle strength recovery [127]. The decline in force generating capacity over the first 2 days of recovery coincide with peaks in soreness (DOMS) which has been attenuated with protein [61, 127] and branched chain amino acid [49] supplementation. Further indirect markers of muscle damage have been shown to be positively affected by protein supplementation with lower peaks of CK 48 h following eccentric exercise alongside the consumption of milk and milk carbohydrate protein supplementation [127, 185]. However not all studies following an eccentric exercise bout showed a suppression in muscle soreness

with protein supplementation compared to a (carbohydrate) placebo condition [59, 77]. Similarly, supplementation with BCAAs did not improve post eccentric exercise recovery of muscle function compared to a placebo [49].

The pro -inflammatory response and elevated reactive oxygen species may promote oxidative protein modifications which impact proteolysis pathways which promote degradation of damaged proteins. As such this has been suggested to facilitate myogenesis and recovery of muscle functional capacity. Heat shock protein (HSP70) expression has risen following eccentric exercise but to a lesser extent with the consumption of a protein supplement (4x 20g casein and whey blend), this could prevent the unfolding of proteins and as such promote less muscle tissue degradation [127]. Similarly NF-κB phosphorylation, activated by increased production of reactive oxygen species, was attenuated with protein supplementation following eccentric exercise [127, 186]. The activation of NF-κB inhibits myogenesis and upregulates pro -inflammatory cytokines (IL-6, IL1β) [127, 186]. Under conditions of NF-κB signalling suppression this is suggestive of a faster transition to an anti-inflammatory state where remodelling can take place alongside accelerated recovery of muscle function [127].

The effectiveness of a protein supplement at improving recovery following strenuous exercise is inconclusive with variable effectiveness [187]. As demonstrated in **Table 1.1** there is a wide variety of study protocols. Supplementation protocols providing exogenous protein in a single dose pre and post eccentric exercise [60] up to 14 d supplementation period [78, 186, 188]. Studies have utilised sedentary, untrained, or recreationally active participants to ensure that the exercise stimulus is novel and unaccustomed [62]. The type of eccentric exercise protocols utilised will impact the extent of muscle damage; with

protocols ranging from 50-300 eccentric isokinetic contractions. Studies that saw an effect of protein supplementation on recovery following eccentric exercise may have induced a greater eccentric exercise stimulus, whereby more damage to the muscle ultrastructure may have occurred, therefore substrate requirements for remodelling were higher. With reductions in strength being 40-70% following elbow flexor eccentric exercise [63, 189] and 35-50% in knee extensors [31, 49, 78, 186, 190], indicating a greater disruption to contractile properties [31, 189], potential necrosis [34] and a greater demand for substrate to remodel the muscle. The magnitude of change in the indirect markers are representative of differing levels of muscle damage, such that a 15-30% decline in muscle function is deemed moderate muscle damage [25]; whereby there is a transient decline in muscle function and an increase in muscle soreness that recovers by 4 days post eccentric exercise alongside no indications of muscle swelling. This is not indicative of necrosis or extreme muscle damage [25, 34, 40, 191] whereby an inflammatory response, ultrastructure damage, subsequent swelling and loss of contractile proteins occurs and therefore additional substrate may be required to assist in the repair and remodelling. Further the differing responses could be due to lack of dietary control over the intervention period, with protein supplementation effectively accelerating the recovery of muscle function when dietary intake was controlled and as such with the additional supplementation daily protein ingestion was higher [78].

Amino acid availability is thought of as being a limiting factor to post exercise recovery. With the provision of sufficient amino acids following eccentric exercise there will be a greater stimulation of muscle protein synthesis and muscle remodelling to alleviate the symptoms of muscle damage and soreness. However

the majority of studies on exercise recovery do not have data, from muscle biopsies, to support this but rely on indirect markers of muscle damage to demonstrate the efficacy of a protein supplement. Direct markers of muscle metabolism and recovery include measurements of satellite cells within skeletal muscle biopsies taken following eccentric exercise, with increases in satellite cell numbers located on type II muscle fibres following eccentric exercise [191, 192] and further increasing with whey protein supplementation [193], this is indicative of expansion of the satellite cell pool (Pax7+) which could be associated with the repair of muscle fibres. However, indirect markers of muscle damage were not different between conditions, suggestive of other processes occurring which assist with recovery from eccentric exercise before myofibrillar remodelling occurs.

Markers of muscle protein synthesis include the phosphorylation of mTOR and p70S6K which as discussed previously are key steps in activating the muscle protein synthetic pathway, therefore phosphorylation of this pathway indicates an up regulation in MPS. Following eccentric exercise an increase in mTOR and p70S6K has been evident, however this does not appear to be influenced by protein nutrition [194]. Following a resistance exercise, the recovery of muscle force generating capacity was accelerated with leucine enriched amino acid mixture (3x 4g EAA), however this was not associated with greater myofibrillar protein synthesis over 96h of recovery [195]. Following eccentric exercise, the daily supplementation of 20g protein blend effectively accelerated the recovery of muscle function however this was in the absence of an elevation in myofibrillar protein synthesis which is at odds with the concept of protein synthesis being required for myofibrillar remodelling and repair damaged myofibrils following

eccentric exercise [78]. This raises questions as to whether protein synthesis is integral to exercise recovery and whether protein supplementation provides any additional benefit to muscle protein metabolism following eccentric exercise, therefore this thesis further aims to explore whether there is an association between MPS and recovery from strenuous exercise.

1.5 Protein nutrition

Dietary protein intake robustly stimulates MPS [90], however this response varies substantially across different exogenous protein sources. Through utilising intrinsically labelled proteins we can understand the metabolic fate of dietary protein by measuring the increase in skeletal muscle bound protein over the early and entire postprandial periods [196]. As discussed previously, amino acids are effective stimuli for signalling pathways that initiate protein synthesis, but they also are the substrates required for this process. The availability of amino acids following a single meal amount of protein (20g casein), is ~55%. These protein derived amino acids become available in the systemic circulation, of this ~11% are directly taken up by the muscle tissue as precursor for *de novo* MPS over a 5h postprandial period [156]. However the remainder of these amino acids are retained in splanchnic circulation, providing precursors for liver protein synthesis. The availability of amino acids for muscle tissue is thought to be dependent upon the total protein dose [168, 169, 197, 198], digestibility and absorption [160, 174, 175, 199], amino acid composition [91, 94, 200], specifically the leucine content [103], and the macronutrient and micronutrient composition (whole food matrix; [176, 201, 202]). Understanding the impact of all these variables on the anabolic response to feeding and exercise in humans is difficult due to the variability in

experimental protocols utilised, however it is well established that skeletal muscle is highly responsive to nutrient intake in healthy adults.

Table 1.1 *Studies investigating recovery after eccentric exercise alongside the ingestion of an exogenous protein source*

Study	Participant characteristic	Design	Exercise	Dietary control	Supplement	Control	Timing	Function measure	Outcome	Additional measures
Buckley, 2010 [59]	Sedentary males (n = 11)	Parallel	Unilateral 100 eccentric isokinetic knee extensors	-	25g WPI 25g WPH	Flavoured water	Immediate, 6h, 22h	Peak isometric torque (1h, 2h, 6h, 24h)	WPH + WPI =	CK = TNFα = VAS =
Cooke, 2010 [188]	Healthy untrained males (n =17)	Parallel	Unilateral eccentric leg press, extension, and flexion (4 x10 @120% 1RM)	-	1.5g/kgBM WPH	Isocaloric CHO	14 days 4 times per day	Isometric and isokinetic knee extensor and flexor strength (1-7, 10 and 14 days)	WPH +	CK = LDH =
Cockburn, 2008 [77]	Healthy male team sports players (n = 24)	Parallel	Bilateral eccentric knee flexors (6 x 10)	-	Milk + CHO Milk	CHO Water	Immediately, 2h	Peak isometric torque and total work (24, 48h)	Milk +CHO + Milk +	CK + Mb + VAS =
Draganidis, 2017 [127]	Recreationally active males (n = 11)	Crossover (6week washout)	Unilateral eccentric contractions of the knee extensors (20 x 15)	-	20g PRO (80% casein, 20% whey) x 4 daily	Isoenergetic Maltodextrin	Immediate, 3h, 6h, 9h, 1x daily	Isometric peak torque (1 x 8 days)	PRO +	CK + VAS + Protein carbonyls = Leucocytes = Proteasome +
Farup, 2014 [193]	Young recreationally active males (n = 24)	Parallel	Maximal isokinetic eccentric- knee extensors (15 x10)	Habitual diet monitored	28g WPH + CHO	Isocaloric CHO	Immediate, 3h, 6h, 9h (3x daily)	Peak isometric torque (24h and 48h)	WP =	CK= VAS = SC +
Ives, 2017 [61]	Relatively sedentary males (n = 60)	Parallel	Unilateral 100 high intensity eccentric -knee extensors	1 day prior and day of study – low antioxidant diet	31g WPH 31g WPH + 100mg berry extract	CHO	Immediately, 6h, 24h	Peak isometric and isokinetic torque -knee extensors (immediate, 1, 2, 6 and 24h)	WPH isokinetic + WPH Isometric =	VAS + TC =

Jackman, 2010 [49]	Non weight trained males (n = 24)	Parallel	Eccentric (12 x10 @120% 1RM)	Full (1.5g/kg)	29.2g BCAA	Non-isocaloric sweetened water	4 x daily (pre and 1h post)	Maximal isometric strength and fatigue (8,24,48,72h)	BCAA =	CK = Mb = VAS + IL6 =
Jameson, 2021 [186]	Healthy recreationally active males and females (n = 18)	Parallel	Unilateral 300 maximal eccentric knee extensors (10 x 30)	Full (1.2g/kg)	20g Protein (whey, pea casein +650mg pomegranate extract) 17g micellar casein + 480mg tart cherry extract	Isocaloric maltodextrin	2 x daily	Maximal isokinetic contractions knee extensors (24, 48h)	PRO +	MPS = VAS = NFκB +
Pavis, 2020 [78]	Healthy recreationally active males and females (n = 18)	Parallel	Unilateral 300 maximal eccentric knee extensors (10 x 30)	Full (1.2g/kg)	20g Protein (whey, pea casein +650mg pomegranate extract)	Isocaloric maltodextrin	1 x7day	Maximal isokinetic contractions knee extensors (7days)	PRO +	CK = VAS+ MPS =
White, 2008 [60]	Sedentary males (n = 27)	Parallel	Unilateral maximal isokinetic eccentric knee extensors (5x10)	-	23g WP +CHO	Water and artificial sweetener	Pre or Post	Peak isometric torque (6, 24, 48, 72, 96h)	WP =	CK = VAS =

= no difference between supplementation and placebo, + source of protein improving indirect markers of recovery with statistical difference between supplementation and placebo
WPH: whey protein hydrolysate, WP: whey protein, BCAA: branched chain amino acids, CHO: carbohydrate
CK: creatine kinase, LDH: lactate dehydrogenase, Mb: myoglobin, VAS: visual analogue scale/ muscle soreness, SC: satellite cells, TC: thigh circumference

1.5.1 Protein dose

It is known there is a dose response relationship between dose of protein and muscle protein synthesis [168, 169], specifically 20g of high quality protein is thought to maximally stimulate MPS and protein intake above this level leads to oxidative catabolism of excess amino acids [169]. The ingestion of a high quality bolus of protein (25g whey protein) immediately following exercise results in greater MPS levels than frequent pulsed doses of protein, this has been alluded to be due to a rapid and pronounced aminoacidemia which is achieved following the bolus dose [203]. Suggesting there is an interplay between quantity and timing of protein ingestion. Majority of studies look at the stimulation of MPS in the acute hours (1-6h) following exercise and the ingestion of a protein source, however MPS returns to basal levels within 2 h after peak aminoacidemia despite continuous AA provision [157, 158], suggesting there is an upper limit of amino acid delivery. This concept is known as the 'muscle full effect' [158] whereby beyond this upper limit there is no longer a use of AA as substrate, with AA being diverted to catabolic processes, evidenced by increased whole body rates of AA oxidation and increases in urea concentrations [168, 169]. This further supports the idea of an optimal dose of protein which is consumed immediately following a bout of exercise which allows maximised MPS responses. However, due to the enhanced sensitivity of muscle post resistance exercise, there may be advantages to repeated boluses to allow an adequate latency period and elicit an optimal amino acid profile for the optimal stimulation of MPS over a 12 h period [204], thereby suggesting that the pattern of protein intake could modify the rate of recovery.

1.5.2 Protein quality

The protein and amino acid composition is used to determine the overall quality of a protein source and its ability to maintain human health. Animal proteins are considered complete proteins due to containing all essential amino acids. All essential amino acids are required to be precursors for muscle protein synthesis [205], with low levels of nitrogen intake, EAA will be used inefficiently to form NEAA due to the metabolic requirement and demand for both essential and non-essential amino acids [206]. EAA only and a balanced mixture of EAA and NEAA have been demonstrated to elicit similar stimulation of MPS demonstrating that NEAA are not necessary for the stimulation of MPS [155].

Protein and amino acid compositions of plant based proteins have been compared to animal based protein isolates [207]. Plant-based proteins have been considered as incomplete due to the lack of one or more essential amino acids, specifically the EAA; lysine, threonine, methionine and cysteine, lending to the widespread assumption that they are not as anabolic as animal based proteins [208]. The amino acids requirements for a complete protein source have been determined by the World Health Organisation and the United Nations University utilising stable isotope studies to provide adequate intake requirements [206]. The understanding of individual amino acids and their metabolic function is broadening the food science and nutrition industry. Currently protein quality is determined using nitrogen measurements, rather than constituent amino acids, using the protein digestibility corrected amino acid score (PCDAAS) and digestible indispensable amino acid score (DIAAS). However the content of nitrogen within a protein source will have differing proportions of amino acids that make up the whole and therefore does not take into consideration the

bioavailability of individual amino acids which would be a step toward understanding the specific quality of a protein source [209].

1.5.3 Digestion and absorption

The availability of dietary amino acids has been shown to be an important regulator of MPS. It has been suggested that the rapidity and magnitude of postprandial aminoacidemia determines the extent of the MPS response. Whereby the peak amino acid concentration, total exposure (area under the curve) and time to peak have been shown to support greater MPS responses. Whey protein is digested rapidly and induces a rapid aminoacidemia and leucinemia and therefore is considered more efficacious than micellar casein or soy protein [160, 174, 175]. It has therefore been suggested that this is the primary nutritional determinant of postexercise MPS. This has been termed the 'leucine trigger hypothesis' which predicts maximal muscle protein synthetic rates from plasma leucine concentrations. It has been suggested that a rapid rise (within ~60–90 min) in plasma leucine concentrations after leucine-rich isolated protein ingestion, and in close temporal proximity to an exercise bout, is optimal for stimulating post-exercise muscle protein synthesis rates [174, 179, 210-214]. When comparing protein sources it has been suggested that casein is unable to increase plasma leucine concentrations to high enough levels to increase MPS to the same extent as whey or soy isolate, due to differing digestion and absorption kinetics and ultimately lower postprandial leucinemia [160, 213]. Leading to more rapidly digested proteins being considered as the most potent in stimulating MPS. By modifying the digestion of these proteins, the rate and magnitude of aminoacidemia can be manipulated and when consumed within close temporal proximity to an exercise bout this can maximise an anabolic

response. Casein protein hydrolysate is accompanied by a greater postprandial amino acid availability and subsequent MPS response compared to an intact casein protein [212]. Such that when consuming a slow releasing protein or a whole food source this impacts the rate of gastric emptying, digestion, absorption, and availability of the constituent amino acids, such that this relationship does not posit a greater anabolic response and are considered sub-optimal MPS responses. However there is growing evidence that the rapidity and magnitude of postprandial aminoacidemia and leucinemia does not predict MPS responses. In recent human studies, whereby a stable isotope infusion, exercise and protein feeding have been utilised there has been a dissociation between these principles. When the casein fraction was modified to induce a more pronounced aminoacidemia compared to its native state (matched amino acid profiles), this altered state did not induce a greater MPS response, as such provided suggestions that the magnitude or timing of the peak in aminoacidemia does not regulate the anabolic response when a high-quality protein source is consumed after resistance exercise [215]. This divergence is evident when comparing milk protein to whey protein isolate, that despite significantly lower plasma leucine concentrations, milk protein resulted in an equally robust anabolic response [216]. Likewise, beef ingestion elicited a greater peak amplitude of leucinemia when compared to skim milk, but did not translate into a greater early (0-2hrs) MPS response [178]. Conversely a slower time to peak plasma leucine concentration elicited a greater anabolic response in whole eggs compared to egg whites [176]. Consuming protein within a whole food matrix is likely to modulate digestion and subsequent aminoacidemia, perhaps obfuscating the importance of the leucine trigger hypothesis when considering whole food sources [211, 217]. Hermans, Senden [218] found no difference between MPS

responses between groups ingesting milk protein or lesser mealworm protein over the early (0-2h) and late (0-5h) postprandial MPS responses, despite milk protein eliciting a greater plasma concentration of EAA and leucine. Taken together these findings suggest that amongst younger individuals the relative protein and leucine intake is only partially predictive of the MPS response. This requires further investigation and will be explored in **Chapter 3**.

1.5.4 Plant based protein nutrition

A plant-based diet is an eating pattern that is dominated by fresh or minimally processed plant foods and the decreased consumption of meat, eggs and dairy products [219]. Plant based proteins comprise a large part of our daily diet and protein intake, however there has been an increase in the demand for a variety of different plant-based protein sources due to an increasing awareness of animal protein derived greenhouse gas emissions and a greater global requirement for protein [220]. Plant derived protein sources have gained popularity and importance when considering health, alongside ethical, animal welfare and environmental concerns [219]. The balance between health, affordability and environment are the key components which form a sustainable diet [221]. Plant based proteins are considered more sustainable due to lower greenhouse gas emissions, energy, land and water consumption [221]. In addition, diets which have a greater consumption of plant-based proteins, are associated with lower body mass indexes and reduced risk of common morbidities such as cardiovascular disease, cancer and type 2 diabetes [222, 223].

Recent advances in the area of plant proteins and muscle protein metabolism has meant that there is an increasing knowledge of anabolic capacities of a wider range of sources. The most common plant-based source to be studied is soya

protein, specifically in comparison to milk or whey protein isolate [160, 173, 197, 224]. The ingestion of soya protein has been shown to have a lower [173, 197] or similar [160, 224] anabolic response to the ingestion of dairy proteins. Due to the lower protein and leucine profiles of plant based proteins [207], consuming higher doses of protein such as 35g wheat protein providing 2.3g leucine induced comparable MPS responses in older males compared to consuming whey protein providing 2.3g leucine [225]. Blending proteins is thought to alleviate the potential issues with deficient plant-based proteins; pulse crops are high in lysine therefore can be used to balance the deficiency in cereal crops for example. As such, these blends can resemble the amino acid profile of a complete animal-derived protein source, with no apparent AA deficiencies. Capitalising on the unique properties of each protein to create a blend; 25% whey, 25% soy, 50% caseinate, has been shown to be capable of prolonging blood aminoacidemia, increasing substrate availability, prolonging signal stimulation of mTORC1 pathways and enhancing MPS, following resistance exercise [182, 226, 227]. However, protein blends which include animal-based proteins are not suitable for individuals who are following a plant-based diet. A blend of wheat-, corn- and pea- derived protein which provided sufficient lysine, methionine, and high levels of leucine (2.4g), supported a MPS response that was equivalent to milk protein concentrate (30g)[228]. Demonstrating that blends can effectively improve the anabolic response compared to the individual protein sources [228]. However, this occurred with differences in the postprandial rise in circulating AA concentrations, increasing the paucity of literature that support the thesis that postprandial amino acid availability may not be the defining factor as to what predicts maximal postexercise postprandial MPS responses. Other examples of plant based protein sources that have adequately maximised MPS at rest and post resistance

exercise are mycoprotein [98], wheat protein [229], meal worms [218] and potato protein [230].

Modifications to overcome these potential differences in anabolic ability, focus on manipulating the digestion and absorption kinetics [212], providing bigger boluses of protein [225], blending different sources together [182, 229, 231] or by matching the leucine content to an animal based protein source [98, 228, 231]. However, limited studies have fortified plant-based protein sources with the deficient EAA. Commonly cereal crops are deficient in lysine [207], the fortification of a wheat and chickpea blend with lysine, effectively stimulated postprandial MPS responses in young adults to a similar extent as a single serving of chicken [232]. The fortification of wheat protein with leucine to match the amount present in whey protein (9.3g leucine) produced a similar postprandial MPS response in an animal model [233].

1.5.5 To pea or not to pea

Pea (*Pisum Sativum*) is a readily available, widely grown and inexpensive pulse crop, that is a good source of starch in the form of carbohydrates, fibre, vitamins and minerals needed for human health, alongside having a protein content of 20-33% [234-236]. Pea protein isolate from the yellow pea, is excellent at improving the nutritional and functional properties of cereals, bakery goods, meat and dairy products/ beverages [234]. Due to its solubility, water and oil holding capacity, emulsifying, foaming and gelling properties [234, 237], it can improve the structure and texture of food. From an allergenic and intolerance viewpoint, pea protein is not classified as a major allergenic and reactions have been limited, alongside being lactose free, gluten free and non-genetically modified organism, offering to be a good plant-based choice [238]. Pea protein has shown to raise

satiety and lower blood glucose concentrations [239], alongside having a high fibre content, providing scope controlling blood glucose regulation, cardiovascular disease and gastrointestinal health [239]. The yellow pea also has antioxidant, antihypertensive and anti-inflammatory properties [237]. Due to these properties it is becoming an increasingly popular protein source.

Pulse proteins are generally high in lysine, leucine, aspartic acid, glutamic acid, arginine however they lack methionine, cysteine and tryptophan [206, 207]. The major proteins found in pulses are globulins and albumins. Globulins make up the majority of the storage protein (65-80%) within a pulse and are made up of two main fractions; legumin and vicilin [234]. Vicilin proteins are highly digestible but have lower concentrations of sulphur containing amino acids and are the target for nutritionally improving pea protein. Vicilin forms amyloids which are protein aggregates, these are resistant to the action of proteases and gastrointestinal digestion which could potentially contribute further to the lack of available sulphur containing amino acids [238]. The albumin fraction is rich in sulphur amino acids but contains antinutritional factors which make it resistance to proteases therefore making the amino acids less bioavailable [240]. These digestibility parameters of a protein ultimately impact the nutritional value. This will be influenced by the extracting and processing conditions to form pulse flours, protein concentrates and isolates [236, 241]. Pea protein isolates will commonly be 80% protein, meet the EAA dose requirements and have sufficient quantities of BCAAs [206, 207], however are below the recommendations for methionine [206]. **Chapter 5** and **6** seek to determine whether the fortification of pea protein with methionine impacts its anabolic capacity and ability to assist with recovery following eccentric exercise.

Our current understanding of pea proteins ability to stimulate muscle protein synthesis and influence functional recovery is limited to a chronic 12 week placebo controlled resistance training programme of the *bicep brachii* [242] and an acute study assessing muscle function recovery over a 4 day period following 90 minutes of whole body eccentric exercise [243]. Babault, Païzis [242] demonstrated that over 12 weeks of resistance training, the daily supplementation of 25g pea protein can increase muscle mass to the same extent as a protein matched whey supplement. This was concluded to be due to both pea and whey proteins having sufficient EAA to maximise muscle protein synthesis, however this study lacked muscle protein synthetic data to support this. Further a whole body 90 minute eccentric exercise protocol was utilised alongside supplementation of three daily doses ($0.9g \cdot kgBM^{-1}$) of whey protein isolate, pea protein isolate or water placebo amongst non-athletic non-obese males [243]. There were no major decrements in performance measures or increases in muscle soreness following the eccentric exercise, subsequently no differences in supplementation were found. There was a significant attenuation in indirect damage markers compared to the water placebo, however due to the lack of an isocaloric placebo to compare the influence of the protein supplements it is hard to draw conclusions from this. To overcome this, the use of a unilateral exercise model, dietary control and an isocaloric placebo can be used to investigate the influence of dietary and exercise interventions on skeletal muscle recovery. However neither of these studies investigated the anabolic ability of pea protein.

The anabolic ability of pea protein has been compared to whey protein ingestion following resistance exercise. Whey protein (1.62g leucine) ingestion induced a

superior aminoacidemia compared to pea protein (1.19g leucine) ingestion however no difference between protein sources were observed in changes to mTORC1 signalling proteins, indicating the stimulation of MPS post exercise being no different between pea protein and whey protein [244]. Most recently, pea protein was demonstrated to support equivalent MPS following a bout of whole body resistance exercise compared to the plant based protein source mycoprotein or a mycoprotein/ pea blend, despite differing postprandial amino acid kinetics [245].

Plant based foods provided in the appropriate amounts and combinations are able to supply adequate nutrients to maintain health and function [246]. The impact of the amino acid profile of a protein source is thought of as the determining factor however due to the nuanced data around postprandial aminoacidemia and muscle protein synthesis this needs to be determined, specifically in relation to the amino acid leucine. **Chapter 3** systematically reviews whether there is an association between leucine and postprandial postexercise MPS response. Subsequently **Chapter 4**, investigates whether providing a sufficient dose of protein and leucine post eccentric exercise accelerates functional recovery in both males and females and if this process is associated with enhanced myofibrillar protein synthesis. Further, there is limited experimental data into non-animal derived protein supplements on recovery from eccentric exercise. **Chapter 0** and **6** seek to determine if a pea protein supplement can improve functional recovery following damage inducing exercise and whether this is supported by elevated rates of muscle protein synthesis over the acute hours immediately post-exercise postprandial as well as over the acute days post -exercise.

2 General Methods

This section focuses on the main methods undertaken throughout this thesis and the key links and features that are consistent throughout the experimental chapters. Further details of each experimental protocol are described within **Chapters 4-7**. **Figure 2.1** is a schematic representation of the experimental time course within this thesis.

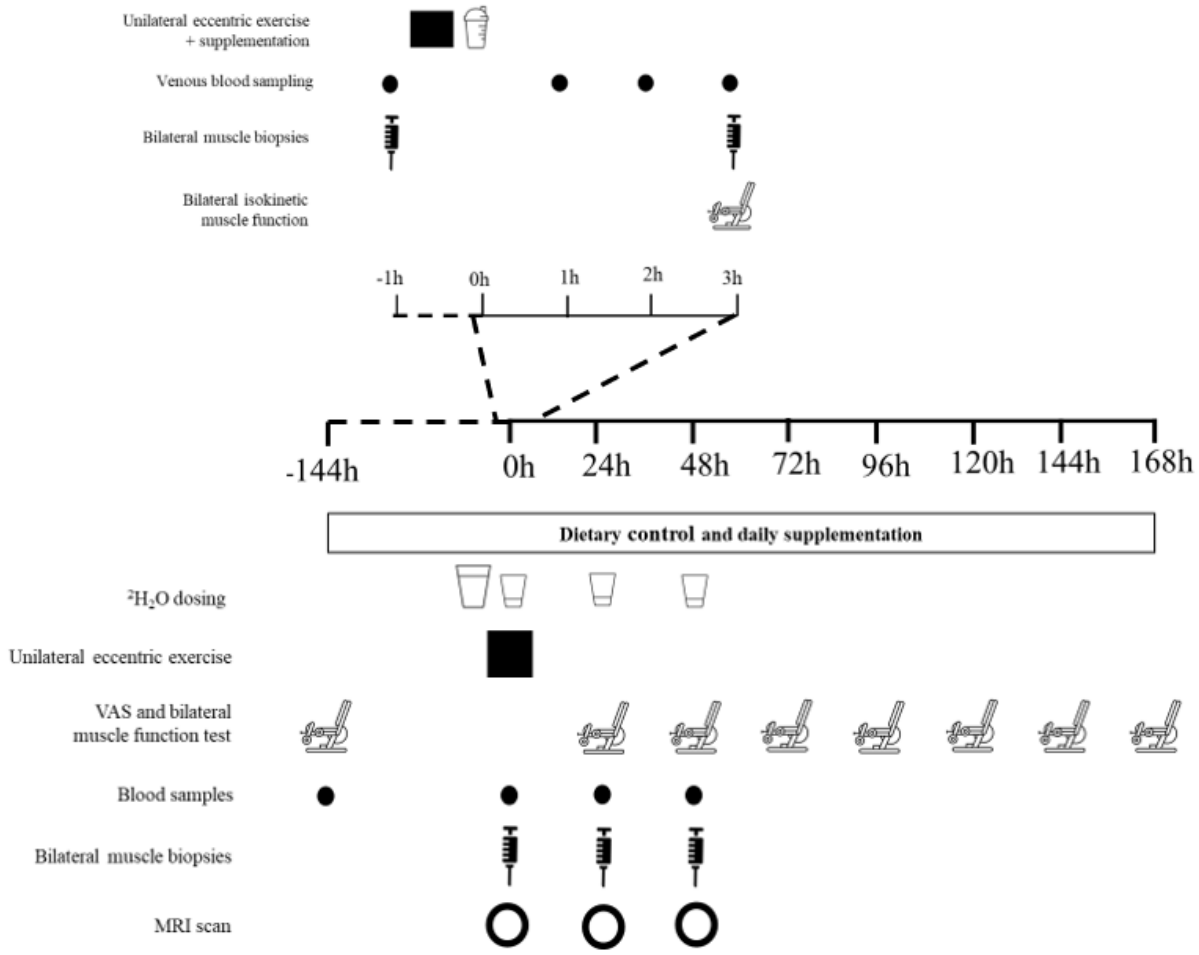

Figure 2.1 *Schematic representation of the studies outlined in this thesis and the sequential time course over which the chapters fit*

2.1 Ethical approval

The studies described in detail within this thesis and shown in **Chapters 4, 0** and **6** were approved by the Sport and Health Sciences ethics committee (Ref No.

161026/B/06, 190703/B/01) in accordance with the Declaration of Helsinki and registered on ClinicalTrials.Gov (NCT02980900, NCT04156386).

2.2 Participant recruitment

Healthy male and female volunteers were recruited through advertisement and word of mouth around the University of Exeter and local area. Potential participants enquired about the study via email and were provided a participant information sheet which provided a lay written explanation of the study. Following reading, if they were still interested in participating in the research, they received a verbal explanation of the study purpose and experimental procedures. They were made aware of any potential risk of taking part and that they were free to withdraw at any time point, this also gave them the opportunity to ask any questions they may have about the research, this was either in person within the Nutritional Physiology Research Unity, St Luke's campus or virtually (implemented to reduce contact during the COVID-19 pandemic). All individuals provided written consent after being provided the verbal and written explanation of the experimental procedures.

Participants were recreationally active, defined as participating in > 2h·wk^{-1} in any form of sporting activity but not engaged in structured exercise training of > 2 sessions per week. Trained individuals, who participated in structured and progressive resistance exercise or high level team sports players were excluded. Participants were deemed healthy based on a medical screening questionnaire and reported the absence of any musculoskeletal injury, medication use, diagnosed metabolic or cardiovascular impairment. Individuals routinely consuming nutritional supplements, non-steroidal anti-inflammatory medication

or consuming <0.8g·kgBM^{-1}·d^{-1} were excluded. Female participants not using an oral contraceptive, took part in the experimental period between days 0-14 of a regular menstrual cycle, specifically day 7 of the experimental protocol falling between days 7-10 of the menstrual cycle (i.e. mid follicular phase). Those who were using oral contraceptives were studied at any time.

From 24h prior to baseline testing and all subsequent test days participants were asked to refrain from vigorous exercise, explained as exercises outside of those reported as a part of their normal exercise routine [247], as documented from the activities recorded on the medical questionnaire during the screening period.

2.3 Exercise protocols

Within **Chapters 4-7**, exercise was performed on an isokinetic dynamometer (Biodex Medical Systems, Shirley, New York, USA)**.** Participants were randomly assigned to eccentrically exercise either their dominant or non-dominant leg in a counterbalanced for leg dominance manner and subsequently the eccentrically exercised leg will be referred to as ECC and the contralateral control leg will be CON. Across **Chapters 4-6,** baseline measures of muscle function were performed on day 1, further muscle function testing was performed on days 7-14 (**Chapter 4** and **0**) with an additional function test performed 3 h following eccentric exercise in **Chapter 6**. This muscle function testing consisted of measurements of muscle soreness and isokinetic torque of the knee extensors.

2.3.1 Unilateral exercise model

In the case of this thesis the unilateral exercise model has been utilised, in all experimental chapters, whereby participants are randomised to complete the eccentric exercise protocol on one leg and the other leg acts as the intra subject

control. This allows the comparison to be made between limbs within a participant. This utilises the assumptions that the two limbs are equally responsive to potential treatment, therefore the responsiveness, in a fasted state, to the contractile stimuli and protein nutrition used throughout this thesis should theoretically be similar across legs [248].

2.3.2 Familiarisation

At least 48 h prior to the first experimental visit all participants attended a familiarisation exercise session at the Nutritional Physiology Research Unit. Participants were seated on the isokinetic dynamometer, straps were placed over the shoulders, hips and thighs to reduce extraneous movement [249]. Seated position was 85° of hip flexion with the lateral epicondyle of the femur aligned with the axis of rotation of the dynamometer and alignment of the knee through the range of motion was adjusted visually by modifying the chair position. A Velcro strap secured the ankle to the dynamometer attachment. The length of the dynamometer attachment was the same for the right and left legs to standardise the moment arm. A muscle function test was completed and five eccentric contractions on each leg were performed. The Biodex configuration for each participant was recorded during the familiarisation for both legs, and this configuration remained the same for sequential tests.

2.3.3 Eccentric exercise protocol

Utilising a unilateral model, maximal voluntary knee extensor eccentric exercise protocol was performed to induce moderate exercise induced muscle damage. Repetitions were completed at an angular velocity of 60°/s through an 80° knee joint range of motion. Participants performed 300 (10 sets of 30 contractions),

each set was separated by 120 s of passive rest. Participants were encouraged to resist the repetition maximally and were provided verbal encouragement. **Figure 2.2** demonstrates the force produced during each eccentric repetition over the 10 sets, which demonstrates the decline in the ability to produce force during this damage inducing exercise protocol. The exercise protocol applied in this thesis has previously been described to effectively induce myofibrillar disruption evidenced by electron microscopy analysis [31] and has effectively induced reductions in muscle function and an inflammatory state [78, 127, 186].

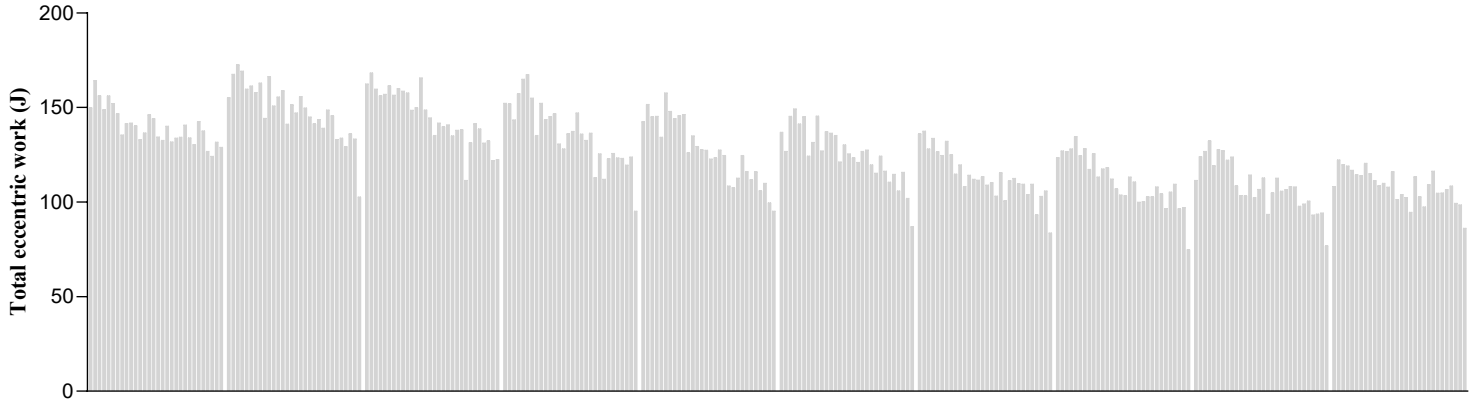

Figure 2.2 *Eccentric work over 10 sets of 30 maximal voluntary contractions performed on an isokinetic dynamometer. Each repetition is an average of all participants This displays the decline in force generating capacity over 10 sets.*

2.3.4 Knee extensor isokinetic work

Isokinetic concentric contractions were performed to determine total isokinetic work. This consisted of 30 voluntary maximal, unilateral isokinetic concentric contractions of the knee extensors. Completed on both the eccentrically exercised and contralateral control legs. Contractions were performed at 75°·sec^{-1} through an 80° range of motion equidistant from voluntary maximal knee extension and flexion and were preceded by a 5-repetition submaximal warm-up. Isokinetic work was determined from the area under the torque time curve.

Muscle function was measured on day 1 at ~08:00am (baseline) and on days 8-14; every subsequent 24h following eccentric exercise. To provide a further exercise stimulus alongside the functional testing on days 8-14 (**Chapter 4** and **0**), participants completed a further 4 sets of 30 repetitions (total 5x30).

Muscle function is the primary marker of contractile ability with this thesis. Muscle function incorporates both peak torque and fatigability of the muscle and as such reflects damage to contractile machinery of the muscle from eccentric exercise [250]. The functional ability of the muscle to produce maximal isokinetic torque during each repetition and the fatigability over 30 repetitions is demonstrated in **Figure 2.3**, with data drawn from the placebo condition across the time points measured in Chapters **5** and **6**. Linear regression analysis demonstrates that over each repetition there is a loss of 1.86Nm at baseline muscle function. Following the bout of eccentric exercise, the ability of the muscle to produce and sustain force production is lower and the reduction in force over each repetition is 1.05, 0.95, 0.97Nm at 3, 24 and 48h following the bout of eccentric exercise.

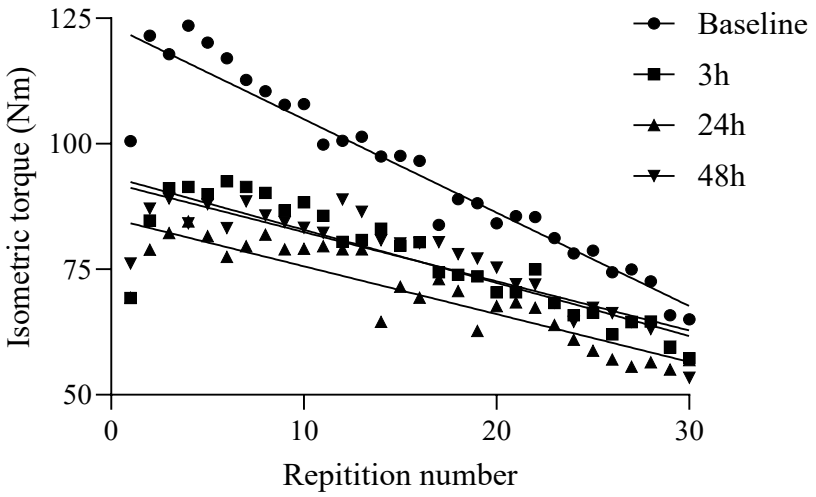

Figure 2.3 *Isometric torque produced with each repetition during the muscle function test performed at baseline and 3, 24 and 48h following the bout of eccentric exercise.*

2.3.5 Muscle soreness

Within **Chapters 4, 0** and **6**; muscle soreness was evaluated using a 100mm visual analogue scale (VAS, **Figure 2.4**) every 24hours after completion of the unilateral maximal eccentric exercise protocol. Participant were asked to use the VAS to rate their muscle soreness in the quadriceps muscle from a sit to stand position. The scale consisted of 100mm line with anchor words at either end of the scale, 'no soreness' (0mm) on the left and 'worst soreness' (100mm) on the right. Subjects placed a mark on the point of the scale that they felt corresponded to their perception of soreness. This mark provided a numerical measure of soreness using the distance (mm) from the left-hand end of the scale to the mark made by the participant. This method is a valid and reliable measure for the measurement of acute pain [251] and has been effectively been utilised to determine the onset of soreness following eccentric exercise [78, 186, 252].

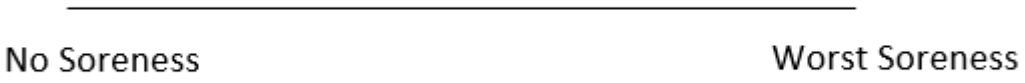

Figure 2.4 *Visual Analogue Scale utilised throughout* ***chapters 4*** *and* ***5*** *to provide a measurement of muscle soreness at baseline and every subsequent 24 h following a bout of eccentric exercise.*

2.4 Diet and supplementation

2.4.1 Supplementation

Drinks were provided by the manufacturer (Beachbody LLC, Santa Monica, California, USA) in sachets which contained a single serving of supplement. These sachets were coded to ensure that double blinding was maintained. Plant based (vegan) pea protein (VGP), whey protein (WHEY), protein blend (PPB; whey, pea and casein) and maltodextrin non-nitrogenous placebo (PLC) supplements were analysed for macronutrient and amino acid composition (Premier Analytical Services, High Wycombe, Buckinghamshire, UK). The nutritional content of the supplements are displayed in **Table 2.1**. A non-nitrogenous placebo was used due to the potential of suboptimal doses of protein or amino acids stimulating MPS [164], such that we wanted to ensure the additional provision of amino acids was the stimulus for exercise recovery. All supplements were prepared and consumed either at home in the morning (~08:00-09:00am, day 2-6; **Chapters 4-7**) or under supervision in the laboratory immediately following exercise (day 1 and 7-14; **Chapters 5** and **7**). Sachets were mixed with 225ml water and the bottle was rinsed with an additional 25ml of water to ensure that all the contents were consumed. All drinks were well tolerated,

consumed within the allotted time (i.e. 5 minutes), and no adverse effects were reported during or after the experimental period.

Table 2.1 *Nutritional Composition of the Supplements*

	PLC (40g)	VGP (40g)	WPI(30g)	PPB (35g)
Macronutrients				
Protein (g/ serving)	0.48	24.84	21.87	20.90
Energy (kJ/ serving)	590.01	620.80	481.20	517.65
Carbohydrate (g/ serving)	31.76	8.40	2.49	7.49
Fat (g/ serving)	0.24	1.24	1.53	0.32
Fibre (g/ serving)	4.04	1.28	1.35	2.98
Amino acid content, g				
Alanine	0.03	1.0	1.22	1.04
Arginine	0.03	1.9	0.54	0.98
Aspartic acid	0.08	2.79	2.71	2.48
Glutamic acid	0.11	3.87	4.20	4.27
Glycine	0.09	0.96	0.44	0.56
Histidine	0.02	0.58	0.38	0.48
Isoleucine	0.02	1.05	1.35	1.33
Leucine	0.05	2.22	2.45	2.33
Lysine	0.04	1.83	2.37	1.99
Methionine	0.02	0.53	0.48	-
Phenylalanine	0.15	1.24	0.72	0.96
Proline	0.03	1.01	1.42	1.37
Serine	0.04	1.32	1.31	1.16
Threonine	0.04	0.88	1.68	1.27
Tryptophan	0.01	0.21	0.44	-
Tyrosine	0.02	0.84	0.68	0.75
Valine	0.03	1.22	1.31	1.36
Total amino acids (g/ serving)	0.73	23.64	24.10	22.31
Essential amino acids (g/ serving)	0.28	9.74	11.16	9.71
BCAA (g/ serving)	0.11	4.48	5.10	5.02

PLC: placebo, VGP: pea protein isolate, WPI, whey protein isolate, PPB, protein blend. PPB analysed separately and two essential amino acids were missing from analysis results

2.4.2 Habitual diet analysis

In all experimental chapters, all participants completed a three-day diet diary (two weekdays and one weekend day) to determine if they met the eligibility criteria for habitual intake of protein. All data was analysed using Nutritics software (Nutritics LTD, Dublin, Ireland). A summation of all participants habitual protein intake involved in the experimental trials within this thesis are presented in **Table 2.2**

Figure 2.5 *Example of controlled diet food preparation for multiple participants*

2.4.3 Dietary control

To ensure that any differences observed would be from the protein supplement, strict dietary control was put in place. Basal metabolic rate (BMR) was estimated using the Henry equation based on age, gender and weight [253] and energy requirements were calculated by multiplying BMR with a 1.6 activity factor. Individual energy requirements were then implemented into a 7 day (**Chapter 6**) or 14 day meal plan (**Chapters 4** and **5**), with all food for breakfast, lunch, dinner and snacks prepared, weighed and packaged into individual boxes corresponding to each day of the week **Figure 2.5**. This was all prepared within

the Nutritional Physiology Research Unit kitchen. Participants were not permitted to consume any other food sources throughout the duration of the controlled diet period, except water was allowed ad libitum. All participants consumed a diet containing 1.2g·kg body mass^{-1} day^{-1} of protein (~15% energy contribution), with the remainder of the diet being ~25% fat and ~60% carbohydrate. All ingredients and instructions for meal preparation were provided along with a log to record compliance (**Appendix** Error! Reference source not found.). Compliance with the d iet was monitored through completed food logs being returned daily, verbal monitoring and the returning of food containers. Summation of the protein intake from the controlled diet including and excluding supplementation are presented in **Table 2.2** from all participants involved in the experimental chapters of this thesis. The control diet was clamped at 1.2g·kg body mass^{-1}·day^{-1} of protein in accordance with recommendations of a daily dose of protein to maintain and support muscle mass [254]. Further from this the addition of a protein supplement took this up to 1.6g·kg body mass^{-1} day^{-1} of protein, which is known to maximise muscle protein anabolism [255].

Table 2.2 *Habitual and controlled diet protein intake across all experimental chapters*

	PLC (*n=18*)	PRO (*n=29*)
Habitual protein intake (g・kgBM^{-1}・day^{-1})	1.24 ±0.43	1.31 ±0.43
Controlled diet protein intake (excluding supplement) (g・kgBM-1・day^{-1})	1.18 ±0.04	1.21 ±0.08
Controlled diet protein intake (including supplement) (g・kgBM-1・day^{-1})	1.20 ±0.03	1.56 ±0.10

Values represented as mean ±SD. PLC; daily maltodextrin placebo, VGP: daily vegan protein supplement. Habitual protein intake recorded with a 3-day food diary. Controlled diet protein intake relative to body mass per day with and without the supplementation.

2.4.4 Deuterated water loading protocol

To measure free living myofibrillar protein synthesis rates, in **Chapters 4, 5** and **6**, an orally administered deuterated water protocol was used. This protocol was designed to achieve body water deuterium enrichment of 0.6%. All participants underwent a one day loading period, whereby participants consumed 6ml·kg body mass^{-1} of deuterated water (70 atom %: Cambridge Isotope Laboratories Inc, Tewksbury, MA, USA) across 8 doses spread over 1.5 hour intervals. The loading doses were spaced out over a day in 1.5h intervals to minimise the risk of participants experiencing symptoms of vertigo and nausea. Thereafter to maintain a steady 0.6% body water ^{2}H labelling participants consumed a daily dose of 0.54ml·kg body mass^{-1} 70% deuterated water upon waking. The dose of deuterated water was relative to body mass as total body water (used as the precursor pool) is influenced by body mass [256] and the dose was chosen due to the assumption that body water pool is ~60-70% body mass in a healthy adult [256]. The top up dose was calculated based on the assumption that body water turns over at 9%·d^{-1} [115].

2.5 Sampling and analysis

2.5.1 Blood sampling

A venous blood sample, from the antecubital vein, via venepuncture, was collected on day 1 from participants when they arrived overnight fasted (~08:00am) and subsequent venous blood samples were collected on day 7 (**Chapter 4, 5** and **6**), 8 (**Chapter 4** and **5**) and 9 (**Chapter 4** and **5**) upon participants arriving overnight fasted and before doing exercise or taking the supplement. During the acute hours post exercise (**Chapter 4**), a Venflon cannula

was inserted anterograde into the antecubital vein of one arm for repeated blood sampling over 3 hours. A 10mL venous blood sample was collected at each time point. Five millilitres of that sample was added into EDTA-containing tubes and centrifuged immediately. The remaining 5mL of blood was added to an additional vacutainer (BD vacutainer LH; BD Diagnostics, Nu-Care, Bedfordshire, UK) and left upright to clot at room temperature for 30 minutes. Blood samples were centrifuged at 3000 x *g* at 4°C for 10 minutes. Blood plasma and serum were aliquoted and frozen at -80°C for subsequent analysis.

2.5.2 Muscle biopsies

All experimental studies (**Chapters 4-6**) involved skeletal muscle sampling during experimental visits. Muscle biopsies were all taken from the *m. vastus lateralis* of the eccentrically exercised leg (ECC) and the control leg (CON) at all time points, utilising the percutaneous needle muscle biopsy technique [257], whilst participants rested in a supine position. The first biopsies were taken in the distal part of the *vastus lateralis* muscle and subsequent biopsies were placed ~2cm proximal to the last insertion, in an attempt to reduce the influence of inflammation from previous biopsies.

The area was shaved and sterilised using iodine solution. Approximately 2.5mL of local anaesthesia (2% lidocaine), was subcutaneously injected, allowing an incision (0.8cm) to be made into the skin and fascia. A 5mm gauge Bergström needle was advanced through the skin and fascia into the muscle where the cutting cylinder was opened, suction applied and then closed, this was repeated several times. Target yield was 50-100mg, once out the leg samples were quickly assessed, any blood or non- muscle tissue were dissected and discarded. The muscle samples were sectioned either being immediately frozen in liquid nitrogen

or frozen in liquid nitrogen cooled isopentane within a minute of excision and stored at -80°C until further analysis. The exact time of biopsy was recorded to calculate the fractional synthetic rate.

2.5.3 Body water deuterium enrichment

Body water deuterium enrichment was measured, using plasma samples collected at baseline, 24 and 48 h post exercise, at the University of Texas Medical Branch. A ThermoFisher Delta V Advantage Isotope Ratio mass spectrometer (IRMS) (Bremen, Germany), equipped with a Finnigan GasBench II (Thermo Fisher Scientific, Waltham, MA, USA), was used for stable hydrogen isotope ratio measurements ($^{2}H/^{1}H$).

2.5.4 Plasma amino acid extractions

Plasma amino acid concentrations (**Chapter 6**; leucine, valine, isoleucine, phenylalanine, lysine, histidine, glycine, glutamic acid, methionine, proline, serine, threonine, tyrosine and alanine) were determined by GC-MS in tert-butyldimethylsilyl derivatives [136]. Plasma samples (500µL) were defrosted at room temperature and deproteinised on ice with 500µL of 15% Sulphosalicylic acid (SSA) and 10µL of internal standard was added. Samples were vortexed and centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was then passed over cation exchange resin columns (100 – 200 mesh; H^{+} form; Dowex 50W-X8; Sigma-Aldrich Company Ltd., Gillingham, UK). Columns were then washed with 1 mL ultra distilled water (ddH_2O), 6ml 0.5 M acetic acid and 5 mL ddH_2O and subsequently eluted with 2 ml 6M ammonium hydroxide (NH_4OH), before being dried under vacuum. The samples were analysed by gas chromatography - mass spectrometry (GC-MS, 7890 GC coupled with a 5975 inert MSD; Agilent

Technologies) in duplicate using electron impact ionization and selected ion monitoring for measurement of isotope ratios.

2.5.5 Plasma cytokine concentrations

In **Chapter 6,** a panel of cytokines were measured from blood plasma collected following eccentric exercise, using a multiplex assay following manufacture instructions and supplied reagents and antibodies (U-PLEX, Meso Scale Diagnostics LLC (MSD) Rockville, Maryland, USA). The assay used biotinylated antibodies coupled to U-PLEX Linkers, which self-assemble onto unique spots on the U-PLEX plate. After analytes in the sample bind to the antibodies, detection antibodies conjugated with electro chemiluminescent labels (MSD GOLD SULFO-TAG) bind to the analytes to complete the assay.

Preparation of the assay included preparing the couple capture antibodies. Involved 200µL of antibody being added to 300µL of linker, vortexed and incubated for 30 minutes. Following incubation 200µL stop solution was added, vortexed and incubated for a further 30 min. This was done for all six chosen cytokines. Next, all the solutions were combined into a single tube, and the total volume was made up to 6mL with stop solution. The prepared U-PLEX 96-well plate was used, 50µL of this solution was added to each well, this was sealed and incubated at room temperate and shaken at 1000rpm for 1 h. The plate was then washed 3 times with wash buffer. Four specific calibrators were provided by the manufacturer, 250µL diluent was added to each calibrator and incubated at room temperature for 1 h, 50µL of each calibrator was added to a diluent and a further 7 standards were made by serial dilution (8 standards). Plasma samples were defrosted and vortexed. Then, 25µL of diluent and 25µL of standard or sample were added in duplicate to the 96-well plate. The plate was incubated and

shaken (750rpm) for 1 h and then washed 3 times. Detection antibody solution was added to each well, sealed and incubated at room temperature for 1h with shaking. The plate was further washed 3 times and buffer (MSD Gold Read buffer) was added to each well. The plate was placed into an MESO QuickPlex SQ 120 instrument (U-PLEX, Meso scale Diagnostics LLC (MSD), Rockville, MD, USA) where the amount of analyte present in the sample is measured against a standard curve to provide the concentration of cytokine.

2.5.6 Myofibrillar Protein-bound [^{2}H] alanine enrichment

The myofibrillar fraction of skeletal muscle tissue was extracted to be able to determine the enrichment of myofibrillar protein-bound [^{2}H] alanine. Myofibrillar protein fractions were isolated from ~30mg wet weight muscle tissue. Tissue was homogenised in 7.5µL·mg^{-1} ice-cold homogenisation buffer (50 mM Tris-HCL pH 7.4, 1 mM EDTA, 1 mM EGTA, 10 mM β-glycerophosphate salt, 50 mM NaF and 0.5 mM activated Na_3VO_4; Sigma-Aldrich Company Ltd.) with a complete protease inhibitor cocktail tablet (1 tablet per 50 mL of the buffer; Roche, West Sussex, UK) using a glass pestle. Homogenate was centrifuged at 2200 x *g* for 10 min at 4°C and the supernatant representing the sarcoplasmic pool was aliquoted off. The remaining protein pellet was washed with 500µL ice cold homogenisation buffer and centrifuged at 700 x *g* for 10min at 4°C. The myofibrillar protein fraction was solubilised in 0.3M NaOH for 30 min at 50 °C, to ensure complete solubilisation the samples were vortexed every 10minutes. Following centrifugation at 10,000 x *g* for 5min at 4°C, the insoluble collagen fraction was removed. The myofibrillar proteins were precipitated in 1M perchloric acid (PCA) and centrifuged at 700 x *g* for 10 min at 4°C. Subsequently the myofibrillar pellet was washed twice with 1ml 70% ethanol and hydrolysed with

2mL 6M HCL at 110°C for 24 h. The samples were then dried under a vacuum, reconstituted in 3mL 25% acetic acid and passed over cation exchange resin columns (100 – 200 mesh; H^+ form; Dowex 50WX8; Sigma-Aldrich Company Ltd., Gillingham, UK) and eluted with 6 M NH_4OH, before being dried again under vacuum. Samples were resuspended in 1 mL distilled water and 1 mL 0.1% formic acid in acetonitrile, centrifuged (10,000 x *g* at 4°C for 3 min), and the supernatant was aliquoted, dried under a vacuum and stored at -20°C. Amino acids were derivatized by adding 50µL *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) + 1% tertbutyl-dimethylchlorosilane and 50 µL acetonitrile and vortexed and heated at 95°C for 40 min [258]. The samples were then transferred to a gas chromatography vial. Alanine enrichment was analysed using a ThermoFisher Delta V Advantage IRMS (Bremen, Germany) fitted with a Trace 1310 gas chromatograph with an online high-temperature thermal conversion oven (HTC) at 1,420°C. The sample (1µL) was injected in splitless mode at an injection port temperature of 250°C. The peaks were resolved on a 30m x 0.25mm ID x 0.25 µm film Agilent Technologies DB-5 capillary column (110°C for 1min; 10°C/min ramp to 180°C; 5°C/min ramp to 220°C; 20°C/min ramp to 300°C; hold for 2min) before pyrolysis. Helium was used as the carrier gas with a constant flow of 1 $mL \cdot min^{-1}$. Any amino acid eluting from the gas chromatograph was converted to H_2 before entry into the IRMS. The enrichment of tracer was measured by monitoring ion masses 2 and 3 to determine the $^2H/^1H$ ratios of myofibrillar protein-bound [^2H]alanine. A series of known standards were applied to assess the linearity of the mass spectrometer.

Myofibrillar protein synthesis rates were calculated (expressed as fractional synthesis rates (FSR)) based on the incorporation of the mean body water

deuterium enrichment as a precursor pool into myofibrillar bound proteins. FSR was calculated using the standard precursor-product method and expressed as hourly or daily rates as follows:

$$FSR\ (\%{\cdot}d^{-1}/\%{\cdot}h^{-1}) = \frac{\Delta E\mathrm{p}}{3.7\ \mathrm{x}\ Eprecursor\ \mathrm{x}\ t}\ X\ 100$$

where Δ*E*p is the increment in [^{2}H]alanine enrichment in myofibrillar protein, expressed as mole percent excess (MPE), between two biopsies, $E_{\mathrm{precursor}}$ is the average body water deuterium enrichment between two biopsies corrected by a factor of 3.7 based upon the deuterium labelling of alanine during *de novo* synthesis, and *t* indicates the tracer incorporation time between two muscle biopsies.

2.6 Magnetic Resonance Imaging (MRI)

Skeletal muscle volume was measured over consecutive days post eccentric exercise in **Chapter 0**. A Siemens 3T Magnetom Prisma MRI scanner (Siemens Medical Solutions, Erlangen, Germany) was used to obtain images of both thighs in the axial plane over the full length of the femur, with the participant lying in supine position. Scans were undertaken at the Mireille Gillings Neuroimaging Centre, Royal Devon and Exeter Hospital, Exeter. Using a 60-channel body coil positioned across both thighs. A T1 Vibe DIXON sequence was used (0.9 x0.9 x1.5 mm resolution, TE 2.46/3.69ms, TR 6.44ms, slice gap 5mm, slice thickness 5mm). During the first scan, a specified distance from a bony landmark was identified and used to align the imaging slices with the same distance used on all subsequent MRI scans to ensure scanning over an equivalent volume. MRI scans were performed on either the baseline visit or day 7 of the experimental protocol (baseline MRI scan) and then the subsequent 24 h (day 8) and 48 h (day 9)

following eccentric exercise. Slicer 4.10.2 software (3D Slicer, National Alliance for Medical Image Computing) was used to analyse images in the axial plane. The anatomical cross sectional area, presented in **Figure 2.6**, of the thigh muscle was calculated by using the thresholding function in Slicer software, with manual erasing to ensure that only the muscle tissue (excluding bone, blood vessels, adipose tissue and skin) was thresholded. Quadriceps specific and total thigh muscle volume were calculated and expressed relative to the control leg (%CON). All analysis was conducted by the same investigator (KW).

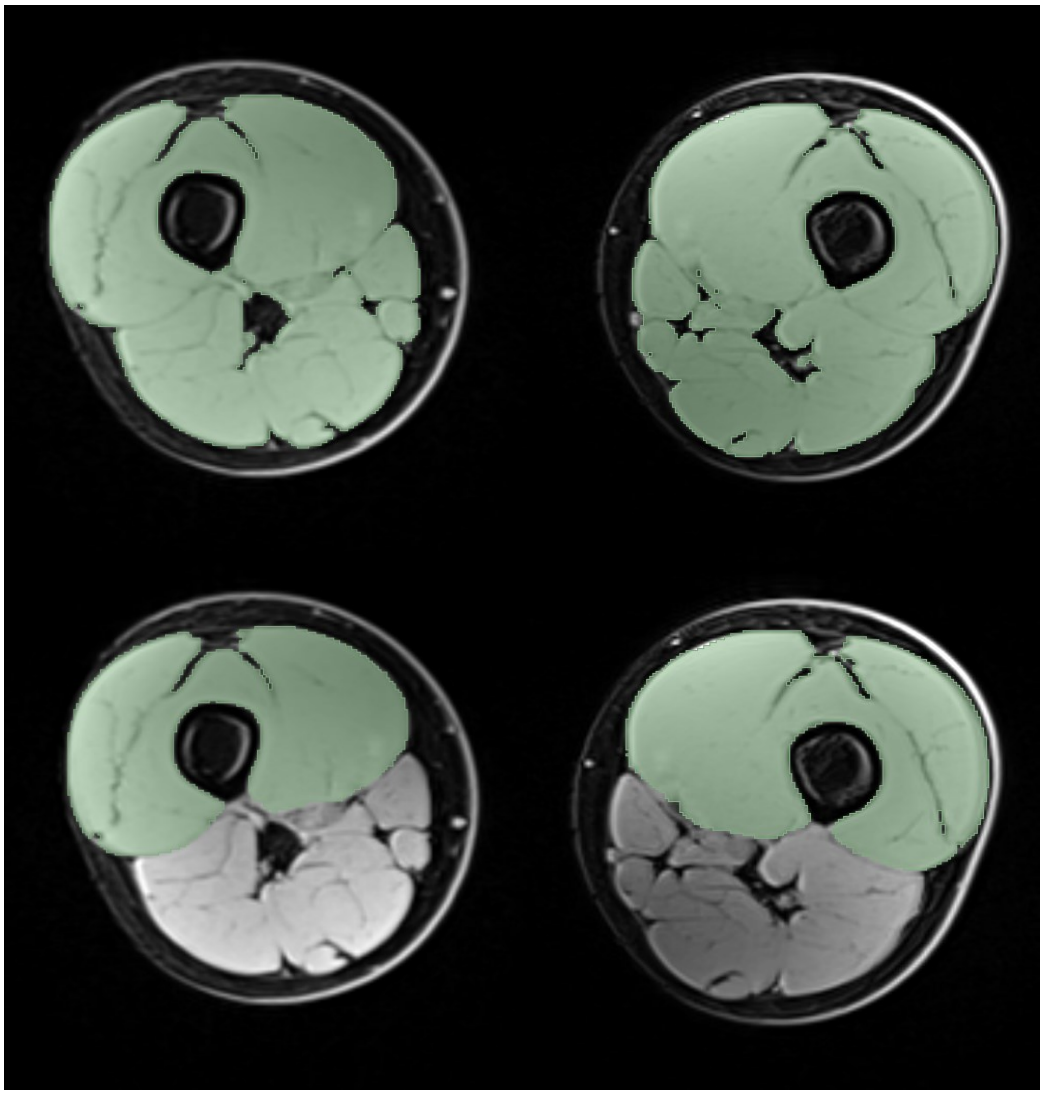

Figure 2.6 *MRI scans of the whole thigh and quadriceps analysed using 3D slicer software to determine muscle volume.*

2.7 Pilot methodology

2.7.1 Fat Fraction

Quadriceps specific intramuscular lipid quantification, we did not measure intramyocellular lipid content at a microscopic level, but images were acquired with a T1 Vibe DIXON sequence. The Dixon technique is used as water and fat molecules will process at different rates. Using ImageJ software [259], we were able to lay over fat only and water only images to provide an image which allowed us to manually draw a region of interest at the same location on the middle slice of the length of the limb across all scans and across participants. Calculation of fat fraction (%) was carried out using the following formula:

$$Fat\ Fraction\ (\%) = \frac{Fat\ Only}{Fat\ only + water\ only}$$

Twenty-three participants MRI scans were analysed prior to, 24 and 48 h following unilateral eccentric exercise across both the eccentrically exercised limb (ECC) and the control limb (CON). **Figure 2.7** demonstrates the intramyocellular lipid concentration following eccentric exercise to detect if lengthening and damaging exercise induced any changes in the extent of fat infiltration into the muscle.

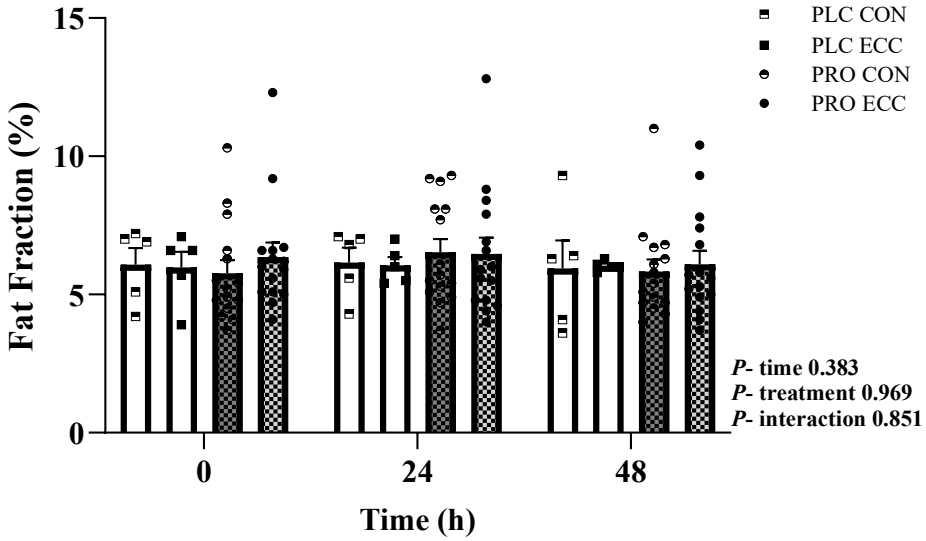

Figure 2.7 *Intramyocellular lipid concentration measured utilising MRI T1 Vibe DIXON sequence to calculate fat infiltration following eccentric exercise.*

2.7.2 Analysing muscle damage using electron microscopy

As discussed in **Section 1.2.3**, morphological damage to the contractile machinery of the muscle is suggested to be the cause of a loss in force generating capacity. However the methodology to determining the said damage is varied and does not quantify what the damage represents. **Appendix**

Table *9.1* shows how the myofibrillar disruption was characterised using electron microscopy following damaging exercise. These characterisations count and determine the areas (expressed in percentages) covered by differing extents of Z -line streaming and disrupted sarcomere structures. However, this does not determine the degree of Z -line or sarcomere disruption. A methodology was proposed whereby disruption could be quantified precisely rather than descriptively categorised and analysed. Sarcomere width was measured to determine the degree of stretch and disruption to the contractile units of the muscle fibres. As well as determining the extent of Z -line disruption with seeking how much streaming/ smearing occurred down the length of a Z -line.

Electron Microscopy analysis

Biopsy sections were fixed by immersion in a solution of 2% glutaraldehyde, 2% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.2). Samples were stored in fixative at 4 °C until further processing. For resin embedment, samples were first washed 3 x 5 min in cacodylate buffer before post-fixation in 1% osmium tetroxide reduced with 1.5% potassium ferrocyanide in cacodylate buffer for 1h at room temperature. After 3 x 5 min washes in deionized water samples were dehydrated in a graded ethanol series (30, 50, 70, 80, 90, 95 and 2 x 100 % ethanol, 10 min per step) then embedded in Spurr resin. 60 nm ultrathin sections were obtained from the resin blocks using an ultramicrotome (Leica Microsystems UC7, Milton Keynes, UK) and collected on pioloform-coated copper mesh grids. The sections were stained in lead citrate before analysis with the transmission electron microscope (JEOL JEM 1400, JEOL, Herts, UK) operated at 120 kV. **Figure 2.8** shows an example of an electron microscopy image analysed.

Sections were sampled in a systematic uniform random fashion by taking 15 images containing profiles of muscle fibres at a nominal magnification of 12.000 X over the entire section for each sample. Images were then analysed using ImageJ software [259]. Utilising the ImageJ software random grids were placed over the images and measurements were taken from the intersect of the grid.

Sarcomere width

A line was placed on an outer border of a Z -line (dark band), this line was then extended down the length of the sarcomere, still following the outer border if the

Z -line deviated, until the next grid line was met. This was then repeated on the adjacent Z -line to the right of the original line measured. Using the measuring tool, the distance between the two lines was measured. This was repeated across 4 grids on each image and repeated across all 15 images per sample.

Z line length

Similar to the sarcomere width, a line was placed on an outer border of a Z -line (dark band), this line was then extended down the length of the sarcomere, but in this instance the line was ended when the border of the Z line was no longer continuous and had been disrupted. The distance measured indicated the extent of smearing/ streaming such that the shorter the distance the more disruption had occurred to the structure of the sarcomere boundary line. This was repeated across 4 grids on each image and repeated across all 15 images per sample.

The utilisation of both, determining the sarcomere width and Z line length, demonstrates that the wider and shorter the measures the greater the extent of disruption. However pilot analysis of 12 samples indicated that there were no differences between the eccentrically exercised leg and the control leg. We were also not happy with the resolution of the images, as areas of the images taken did not demonstrate the clear structure of the muscle such that measurements could not be taken.

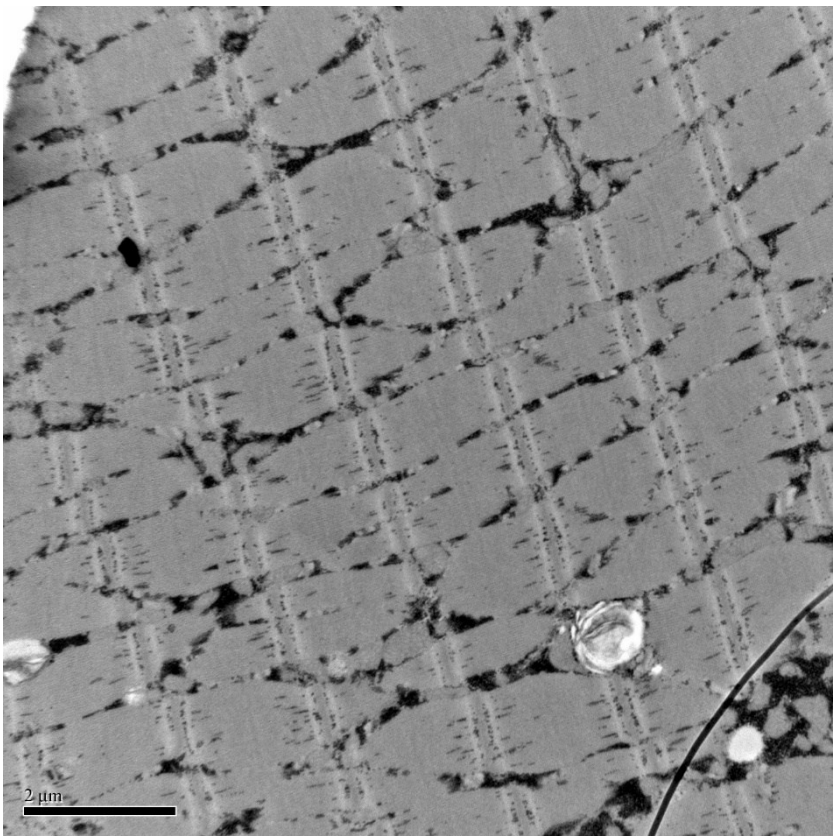

Figure 2.8 *Electron microscopy image of a muscle sample*

3 Association of postprandial post-exercise muscle protein synthesis rates with dietary leucine: a systematic review

'If we knew what it was we were doing, it would not be called research, would it?'

Albert Einstein

The work contained in this chapter is published

Wilkinson, K., Koscien, C. P., Monteyne, A. J., Wall, B. T., & Stephens, F. B. (2023). Association of postprandial postexercise muscle protein synthesis rates with dietary leucine: A systematic review. *Physiological Reports*, *11*(15).

3.1 Introduction

Prolonged resistance exercise training increases skeletal muscle mass and strength, advantageous adaptive responses to support athletic/health goals in a range of individuals [41, 260]. Training induced increases in muscle mass are mechanistically underpinned by persistent periods of positive net protein balance, and therefore protein accretion, within muscle tissue [91]. A single bout of resistance exercise stimulates muscle protein synthesis (MPS) rates, peaking in the immediate hours subsequent (~2-6 h; [261, 262]) and remaining elevated for ~24-48 h [87, 88, 263]. However, resistance exercise also stimulates muscle protein breakdown (MPB) rates such that, in the postabsorptive state, muscle protein net balance remains negative [87]. As a result, nutrition plays a vital role in promoting post-exercise net positive protein balance in muscle and, therefore, muscle reconditioning.

Protein ingestion before [264, 265], immediately after [160, 161, 210, 266] and up to 24 h [201, 267, 268] post resistance exercise augments the rise in MPS rates and, albeit less potently, inhibits MPB rates [93] resulting in a positive net muscle protein balance. The magnitude of the (post-exercise) MPS response to protein ingestion appears to increase in a dose-response manner plateauing somewhere between 20-40 g [161, 168, 269], a relationship that shifts to the right in more anabolically insensitive older adults [82, 162, 270, 271]. However, as opposed to total protein *per se*, this relationship has been suggested to be more contingent on essential amino acids [90, 91, 162, 200] and, in particular, leucine [167, 272], which has a well characterized molecular role in stimulating the mTORC1/P70S6K signalling pathway (the major myocellular anabolic cascade; [103, 107, 171, 273]). However, the nature of the relationship between leucine

and postprandial MPS (particularly when considered in the post-exercise phase) remains to be fully defined.

Some reports imply that simply the amount of leucine contained within the ingested food/meal directly dictates postprandial MPS rates (i.e. 'leucine threshold' concept) [159]. This pragmatic dose-response view allows simple recommendations to be made, such as ~2 to ~3 g to be consumed for measurable and optimal post-exercise MPS responses, respectively [88, 274]. Other reports take account of physiological variables such as protein digestion and amino acid absorption which, together, dictate peripheral leucine availability following protein ingestion and, thus, a stimulus actually seen by the muscle (i.e. 'leucine trigger' concept; [160, 275]). However, even within this more sophisticated view, it is unclear whether the peak concentration [160, 275], rate of rise [275, 276], or total postprandial availability of plasma [277] (or even intramuscular unbound) leucine is the prime 'trigger'. A recent qualitative systematic review [217] supported the utility of the leucine trigger hypothesis within muscle of older individuals and during studies where crystalline amino acid mixtures or isolated proteins were ingested. The predictive value of the hypothesis diminished in younger subjects and/or where protein-rich whole foods (within their unique matrices) [176, 178, 267, 278] and/or mixed meals [201, 279] were ingested. However, the definition of the leucine trigger in this review was binary and defined only as a "greater overall plasma leucine response" which did not allow for any quantitative dose-response relationship to be established.

We conducted a quantitative systematic review with the primary aim of refining our understanding of the relationship between ingested leucine and the magnitude of postprandial post-exercise MPS rates. We compiled data from

human studies that applied a study design including bolus ingestion of amino acids or protein (either alone or contained within a food/meal) and the execution of a single resistance exercise bout, combined with the parallel measurements of MPS rates, and with further inclusion criteria applied around postprandial plasma leucine concentrations. We clearly demarcated between the leucine threshold hypothesis (i.e. leucine dose), and the three distinct (sub)variables identified within the leucine trigger hypothesis (i.e. peak magnitude, rate of rise and total availability of plasma leucine) and evaluated their relationships with the magnitude of postprandial post-exercise MPS responses in young and older adults.

3.2 Methods

This study was registered on, and the protocol was uploaded to, PROSPERO (CRD42021227295). The review was conducted based on PRISMA guidelines 2020, in line with quantitative systematic reviews [280]. The primary outcome of this systematic review was to refine our understanding of the relationship between ingested leucine and the magnitude of postprandial post-exercise MPS rates. Heterogeneity in the absolute determination of muscle protein synthesis (MPS) rates and plasma leucine concentrations between studies, methods and laboratories, was accounted for by assessing delta changes on both variables (MPS and plasma leucine concentrations) as our primary depiction of the data.

3.2.1 Search strategy

A systematic search of the literature was conducted in Medline, Cochrane, and Embase databases on the 12th July 2022. The search was limited to human studies written in the English Language. The medical subject headings (MeSH) “Leucine”, “Protein Biosynthesis”, “Dietary Supplements”, “Dietary Proteins” and

“Muscle Proteins” were utilised. Boolean operators “AND” and “OR” were used to combine search terms. The following search terms were used; (protein OR leucine OR amino acid OR supplement* OR diet* OR consume* OR intake* OR ingest* OR powder OR drink* OR shake OR isolate) AND (muscle* OR myofibrillar* OR mixed OR muscular OR protein synthesis) AND (weight* OR resistance* OR strength OR isometric OR train* OR exercise OR lift*) AND (randomized OR randomized control trial). No publication date restrictions were put in place.

3.2.2 Eligibility criteria

All randomised controlled trials (RCT) reporting MPS rates in healthy human adults after bolus ingestion of an amino acid, amino acid mixture, isolated protein source, protein containing whole food, or mixed meal in close temporal proximity (maximally 1 h before or after) to a bout of resistance exercise (exercise against an external load) were considered for inclusion. Resistance exercise protocols was considered broadly, with a variety of exercise protocols included, differing in modalities and mechanics of movement. All these protocols were designed and considered to provide a maximal stimulus and therefore we assume that this broad study inclusion allowed the examination of a ‘maximal’ exercise induced stimulation of MPS and the further examination of how this is modulated by leucine consumption.

Further inclusion was applied whereby studies had to report plasma leucine concentrations for at least 1.5 hours after protein ingestion with time intervals of, at most, 30 minutes to calculate peak plasma leucine magnitude, rate of rise to peak plasma leucine magnitude, and total postprandial plasma leucine availability. MPS rates needed to be determined by the primed continuous

infusion of stable isotopically labelled amino acid(s) (though specific isotope or in which amino acid it was labelled was not required) and plasma leucine concentrations via venous or arterialized-venous blood sampling methods. With quantification of amino acid concentrations via gas chromatography - mass spectrometry (GC-MS).

3.2.3 Exclusion Criteria

Studies that were excluded were: those where participants were classified as unhealthy; if a source of protein was provided via intravenous infusion or repeated doses (as opposed to a single bolus); if more than one resistance exercise session was performed; where the protein bolus was provided more than 1 h pre or post- exercise; or, if an acute measurement period of MPS was not available.

3.2.4 Data collection

Two reviewers (KW and CPK) screened all titles and abstracts to identify potentially eligible studies, and full papers were obtained and assessed for inclusion independently by these authors. Any disagreement regarding eligibility was resolved through deliberation or referred to a third-party author (AJM), to resolve the decision, if necessary. All duplicates were identified and removed. Automation tools within the Rayyan software were used to filter key words, to detect studies that did not fit the inclusion criteria (i.e. study populations in rats, pigs, children).

3.2.5 Data extraction

Predetermined relevant outcome variables from each study were extracted by one reviewer (KW) and the other reviewer (CPK) revisited all to check for discrepancies. Relevant variables included: number of participants, participant

characteristics (age, sex, and training status, if supplied), protein supplementation protocol (protein dose and leucine dose), exercise intervention, mixed muscle or myofibrillar protein synthesis values and plasma leucine concentrations. If protein or leucine doses were given relative to body weight this was calculated with the mean body weight of the participants in that group to normalise all data to absolute doses. If the study in question did not provide the leucine dose within the nutritional content provided for the protein source then manufacturer information was searched [143, 145, 175, 215, 281, 282] or corresponding authors were contacted to provide manufacturer information [283-285] or nutritional analysis [268]. If the study included more than one protein source (e.g., whey and soy), outcome measures were taken for both and treated as separate study arms. If the study included a placebo or control group that was not a protein source this was not included in data extraction. Protein sources with additional fortification and co-ingestion with macronutrients were noted and included. Data were further characterised into whole food sources (mixed macronutrient non- supplemental protein) and non- whole food sources. This is represented visually within the presented data set herein. Data were considered as a whole data set, and then organised into young and older participants. This was based on the descriptive statistics of the participants provided. The mean age of the younger participants within a given study was required to be between 18 and 40 y. The mean age of the older participants within a given study was required to be >55 y. This was to ensure that the threshold for the onset of age related sarcopenia had been met [286]. Where numeric data was not reported in tables or text, corresponding authors were contacted to provide further details. If authors could not be reached, data was extracted from charts and figures using Web Plot Digitizer (V.3.11. Texas, USA: Ankit Rohatgi, 2017).

3.2.6 Risk of Bias

The Cochrane Handbook and tools [287, 288] were used for the risk of bias assessment for each individual study. The quality of each study was assessed by one reviewer (KW) and checked by another reviewer (CK), any disagreement was resolved through deliberation between KW and CK. Six main criteria were assessed, and the quality of each study was based on high, low or unclear risk of bias (Appendix **Table 9.2**). Studies with a high risk of bias were due to blinding procedures, usually in the case of whole food protein sources [278, 279], where blinding of the allocated intervention was not possible. Sequence generation was considered a high risk of bias when allocation to the intervention was based on a criterion such as younger or older participants therefore assignment to the intervention was non-random. Both studies with high risk of bias variables were considered satisfactory for this data set.

3.2.7 Synthesis methods

The main outcome variables used in this review have been converted from the data extracted and have been used to visually display the data in the figures. Basal [postabsorptive] fractional synthetic rate (FSR) *and* post-exercise postprandial FSR (%·h^{-1}) were used to calculate delta change (ΔFSR (%)) for normalisation across studies. These were then split into early (0-2 h post-exercise and/or protein ingestion) and the entire postprandial (0-6 h post-exercise and protein ingestion) MPS response. Plasma leucine concentrations were displayed as peak plasma leucine concentration (highest single mean value reported), rate of rise to peak plasma leucine magnitude (peak plasma leucine concentration minus basal plasma leucine concentration divided by time in minutes to peak plasma leucine concentration) and total postprandial plasma leucine availability

(incremental area under the curve (iAUC/180 min)). Data were analysed using linear regression; coefficient of determination (r^2), significance (*P* value) and y intercept (b_0) have been presented for interpretation. Subject characteristics are presented as mean ± standard deviation.

3.3 Results

3.3.1 Literature search and study inclusion

Figure 3.1 shows the process of article selection with 38 studies ultimately included. Within these 38 studies there were 77 study arms (i.e. total number of eligible intervention groups), with a total sample size of 711, to determine aspects relating to the leucine threshold hypothesis (**Appendix *Table 9.3*** [98, 101, 143, 145, 171, 176, 178, 182, 213, 214, 218, 230, 231, 262, 266, 277-279, 281-285, 289-303]). Studies which met all the inclusion criteria except not taking a basal muscle biopsy, (i.e. [304]) were excluded in order to calculate delta change from basal MPS.

Of these 38 identified RCTs, 6 studies were crossover in design [176, 178, 266, 284, 297, 302], whereas the remainder were parallel design. Double blinding procedures were used in 11 of the 38 studies, 5 were single blinded and the remainder were unblinded. Further exclusion was applied, whereby 7 studies did not meet the criteria of providing plasma leucine concentrations, leaving 31 studies (61 study arms) included in the further analysis to determine aspects of the leucine trigger hypothesis (Supplementary Information).

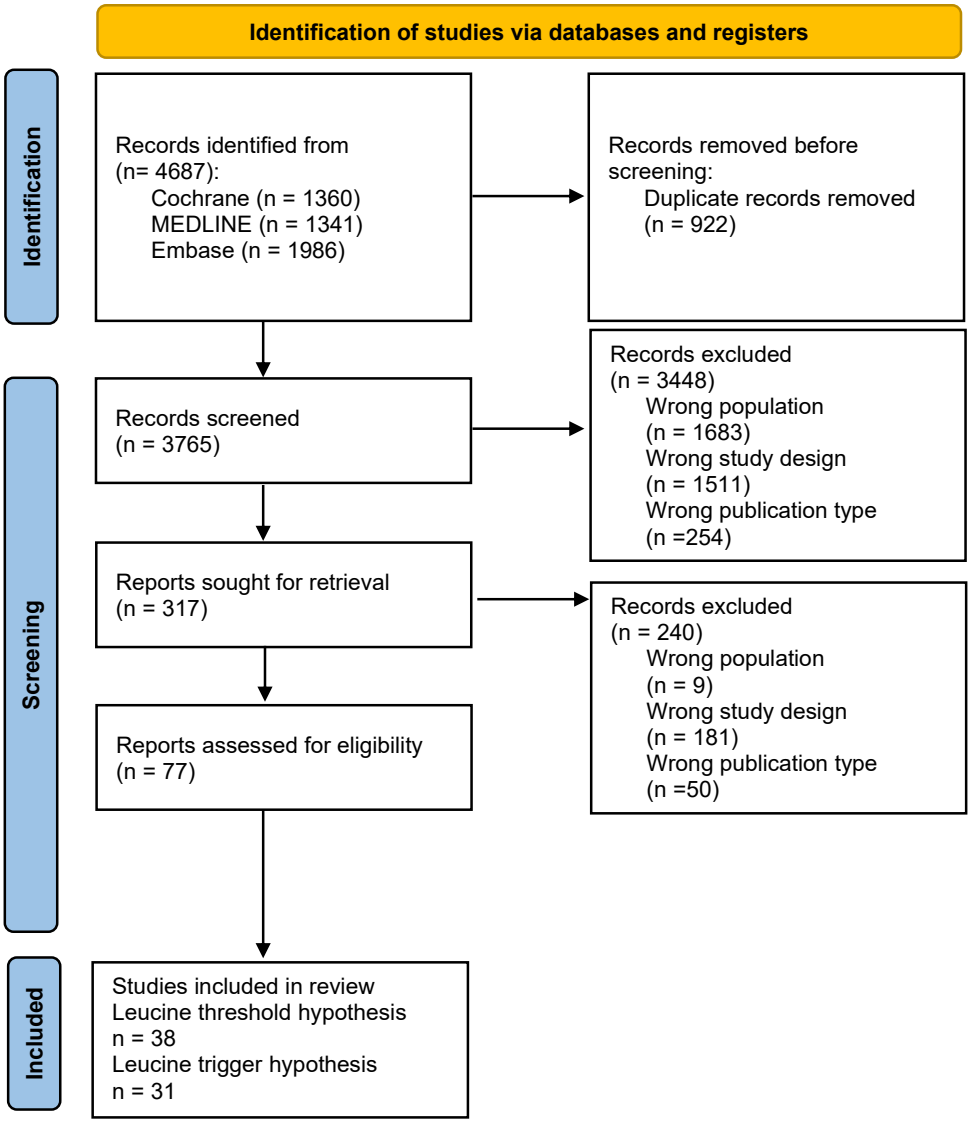

Figure 3.1 *Flow diagram of the screening process in accordance with the PRISMA guidelines*

3.3.2 Participants' characteristics

Of the total 38 studies, 5 of the studies recruited females only [277, 290, 293, 294, 303] and 6 recruited males and females [231, 278, 279, 284, 296, 299], with the remainder in males only. The 77 study arms relating to the leucine threshold analysis, 45 studies were of younger adults and 32 of older adults. The age range of younger participants was 19-29 y (23 ±2.3 y, 409 male and 40 female

participants), and the age range of older adults was 57-74 y (68 ±3.5 y, 142 male and 106 female participants). The 61 study arms relating to the leucine trigger analysis, 35 were studies of younger adults and 26 of older adults. Eleven study arms defined their participants as resistance trained individuals, whilst 19 study arms stated the participants were recreationally active. Further, 6 study arms recruited low to moderately active participants with the remainder of the study arms reporting healthy individuals with no specific training status provided.

3.3.3 Protein sources

Bolus doses of orally administered isolated proteins comprised: whey (33 study arms), casein (3 study arms), milk protein concentrate (10 study arms), crystalline essential amino acid mixtures (9 study arms) and isolated protein blends (4 study arms). Other sources included protein rich foods: pork (2 study arms [278]), beef (3 study arms [178, 279]), mycoprotein (2 study arms [98, 300]), protein rich meal replacements (4 study arms[289]), cheese [298], meal worms [218], collagen protein, potato protein [230], egg white [176] and egg yolk [176], all with one study arm each.

3.3.4 Resistance exercise interventions

A unilateral exercise model was used for 27 out of the 38 studies, with the remaining 11 studies using a bilateral exercise model. The exercise protocols consisted of a variety of reps and sets ranging from 1-10 sets and 8-36 reps or to volitional exhaustion/failure. All studies had a familiarisation with the exercise equipment and tested for maximum strength to determine the workload. The intensity ranged from 16 -90% of 1 repetition maximum (RM). Within 3 study arms maximal leg extension exercise was on a dynamometer [98, 300]. The exercise protocol was either leg press (1 study arm [284]), leg/ knee extension (54 study

arms), both leg press and leg extension (20 study arms), arm cable curl (elbow flexion) (1 study arm [302]) or combination of upper and lower body resistance exercise session (1 study arms [302]).

3.3.5 Postprandial post-exercise periods

The measurement of MPS was taken within the mixed-muscle protein fraction for 4 studies [98, 218, 297, 300] and the remaining studies measured MPS in myofibrillar proteins. The basal biopsy was conducted pre-intervention and the incorporation period for the measurement of MPS ranged from 1.5 to 6 h postprandial, post-exercise. All studies collected muscle biopsy tissue from the *m. vastus lateralis*, except 1 study with measurements of MPS from tissue collected from the *biceps brachii* [302].

3.3.6 Leucine dose

A graphical depiction of the relationship between ingested leucine dose and the delta change in postprandial, post-exercise MPS rates is represented in **Figure 3.2** with data illustrated as an early (0-2 h) and entire measurement (> 2 h) phase, and presented as an entire dataset (**A**), and for young (**B**) and older (**C**) adults separately. When considering the entire dataset (**Figure 3.2A**), leucine dose showed no relationship to delta change in post-exercise MPS rates over the early phase (r^2 = 0.03, *P* = 0.33, b_0 = 78.43), but a significant correlation was observed over the entire measurement period (r^2 = 0.05, *P* = 0.03, b_0 = 76.2). The latter was mainly driven by data obtained from older adults, given divergent responses between the age categories were observed. Specifically, the relationship between leucine dose and postprandial post-exercise MPS change was not present in young adults (**Figure 3.2B**) over either the early (r^2 = 0.006, *P* = 0.74, b_0 = 118.5) or entire (r^2 = 0.01, *P* = 0.51, b_0 = 108.1) measurement periods,

whereas correlations were observed over both periods (r^2 = 0.64, *P* = 0.02, b_0 = 7.64 and r^2 = 0.18, *P* = 0.01, b_0 = 42.33 over the early and entire measurements periods, respectively) in older adults (**Figure 3.2C**).

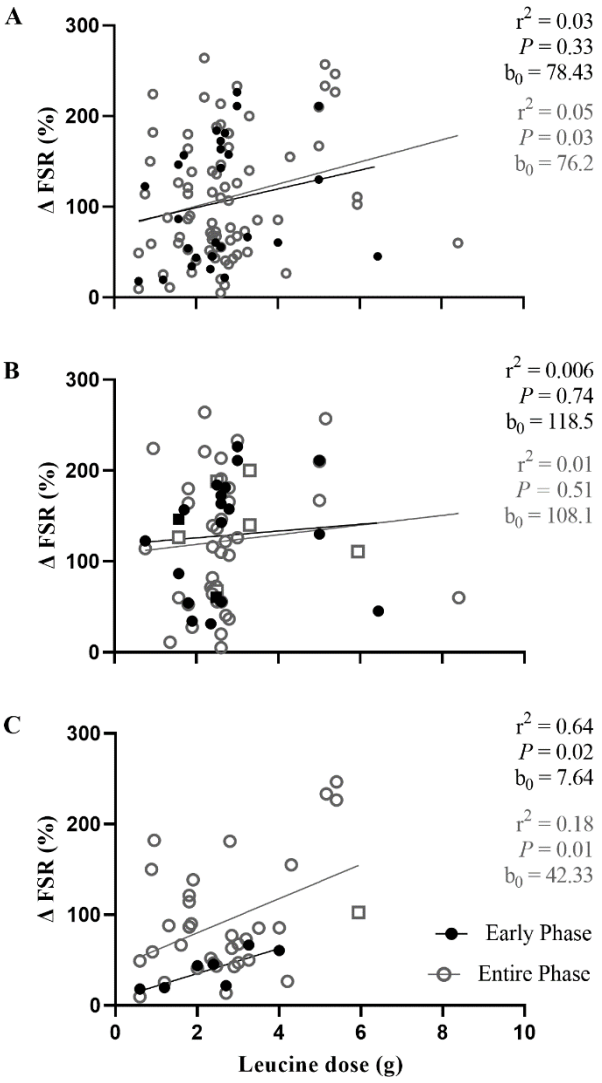

Figure 3.2 *Delta change (postprandial post-exercise increase) in muscle protein synthesis rates (MPS), early (0 - 2 h) and during the entirety of the postprandial period, expressed as fractional synthetic rate (FSR), in response to a leucine dose in all participants (77 study arms)* ***(A),*** *young participants (45 study arms; 19-29 years) (**B**) and older participants (32 study arms; 57-74years) (**C**). Squares represent whole food sources. Data were analysed by linear regression; coefficient of determination (r^2),* P *value and y- intercept (b_0) are presented.*

3.3.7 Leucine trigger hypothesis

A graphical depiction of the relationship between ingested leucine dose and the peak magnitude, rate of rise and total postprandial availability of plasma leucine are represented in **Figure 3.3, Figure 3.4** and **Figure 3.5**, respectively. With data presented as an entire dataset (**A**), and for young (**B**) and older (**C**) adults separately. Further graphical depiction of the relationship between delta change in postprandial, post-exercise MPS and peak magnitude, rate of rise and total postprandial availability of plasma leucine are represented as an entire dataset (**D**), and for young (**E**) and older (**F**) adults separately.

3.3.8 Peak plasma leucine magnitude

When considering the entire data set (**Figure 3.3A**), the leucine dose showed a relationship with peak plasma leucine magnitude (r^2 = 0.27, *P* = <0.0001, b_0 = 138.4). With a significant relationship observed between leucine dose and peak plasma leucine magnitude for both younger (**Figure 3.3B**; r^2 = 0.47, *P* = <0.0001, b_0 = 59.57) and older (**Figure 3.3C**; r^2 = 0.24, *P* = 0.01, b_0 = 209.9) adults. However, peak plasma leucine magnitude showed no relationship to delta change in postprandial, post-exercise exercise MPS rates when considering the whole data set (**Figure 3.3D**; r^2 = 0.02, *P* = 0.18, b_0 = 123.7) and this relationship was not altered when split for age; leucine dose showed no relationship to delta change in post-exercise MPS rates amongst the younger (**Figure 3.3E**; r^2 = 0.00001, *P* = 0.99, b_0 = 121.5) or older (**Figure 3.3F**; r^2 = 0.03, *P* = 0.35, b_0 = 99.52) adults.

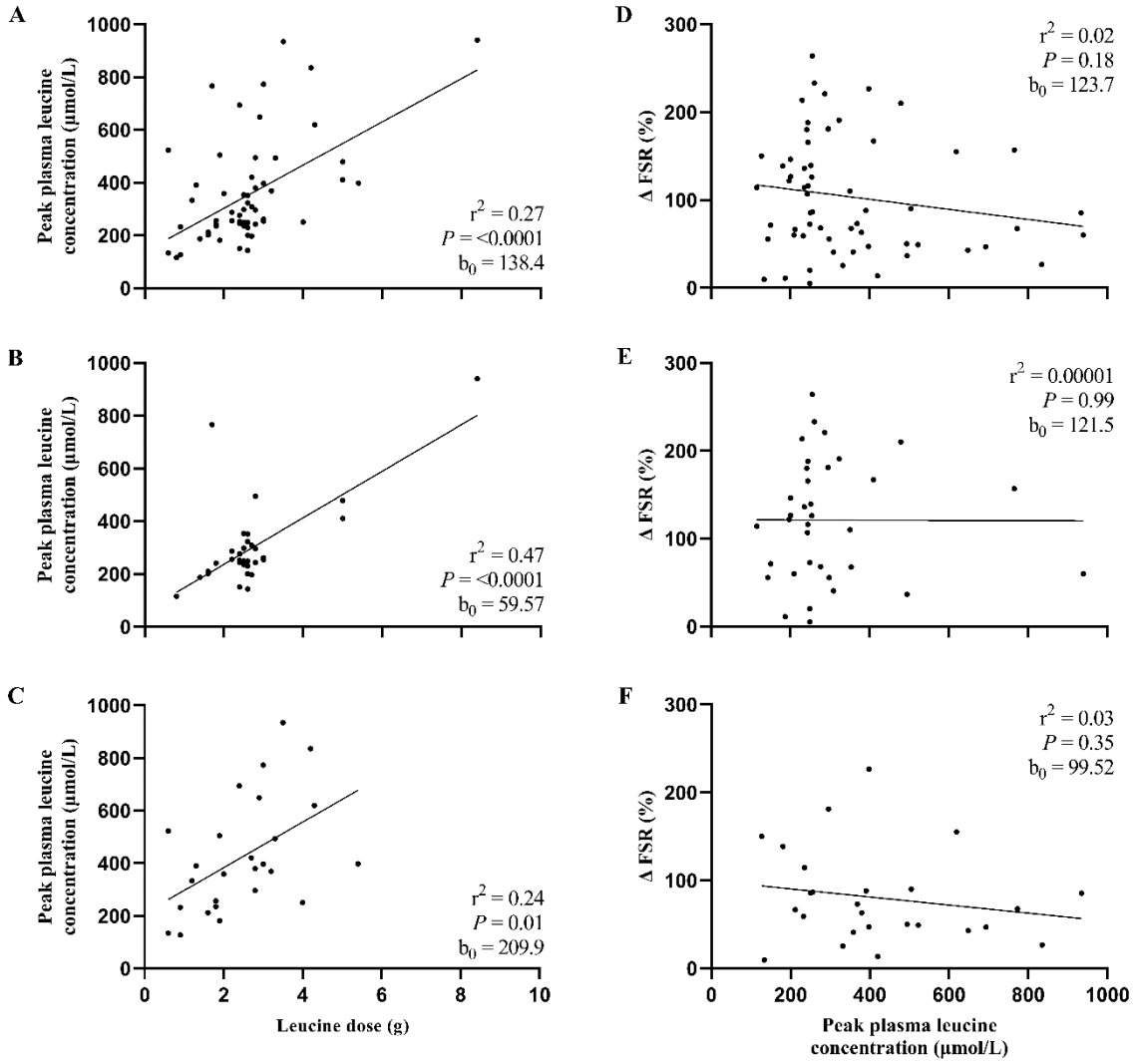

Figure 3.3 *Peak plasma leucine magnitude (highest mean value reported) in response to a leucine dose provided as a bolus protein ingestion within an hour of resistance exercise in all participants (61 study arms)* ***(A),*** *young participants (35 study arms; 19-29 years) (**B**) and older participants (26 study arms; 57-74years) (**C**). Delta change (postprandial post-exercise increase) in muscle protein synthesis rates (MPS), expressed as fractional synthetic rate (FSR) in relation to peak plasma leucine magnitude in all participants* ***(D),*** *young participants (**E**) and older participants (**F**). Data were analysed by linear regression; coefficient of determination (r^2),* P *value and y- intercept (b_0) are presented.*

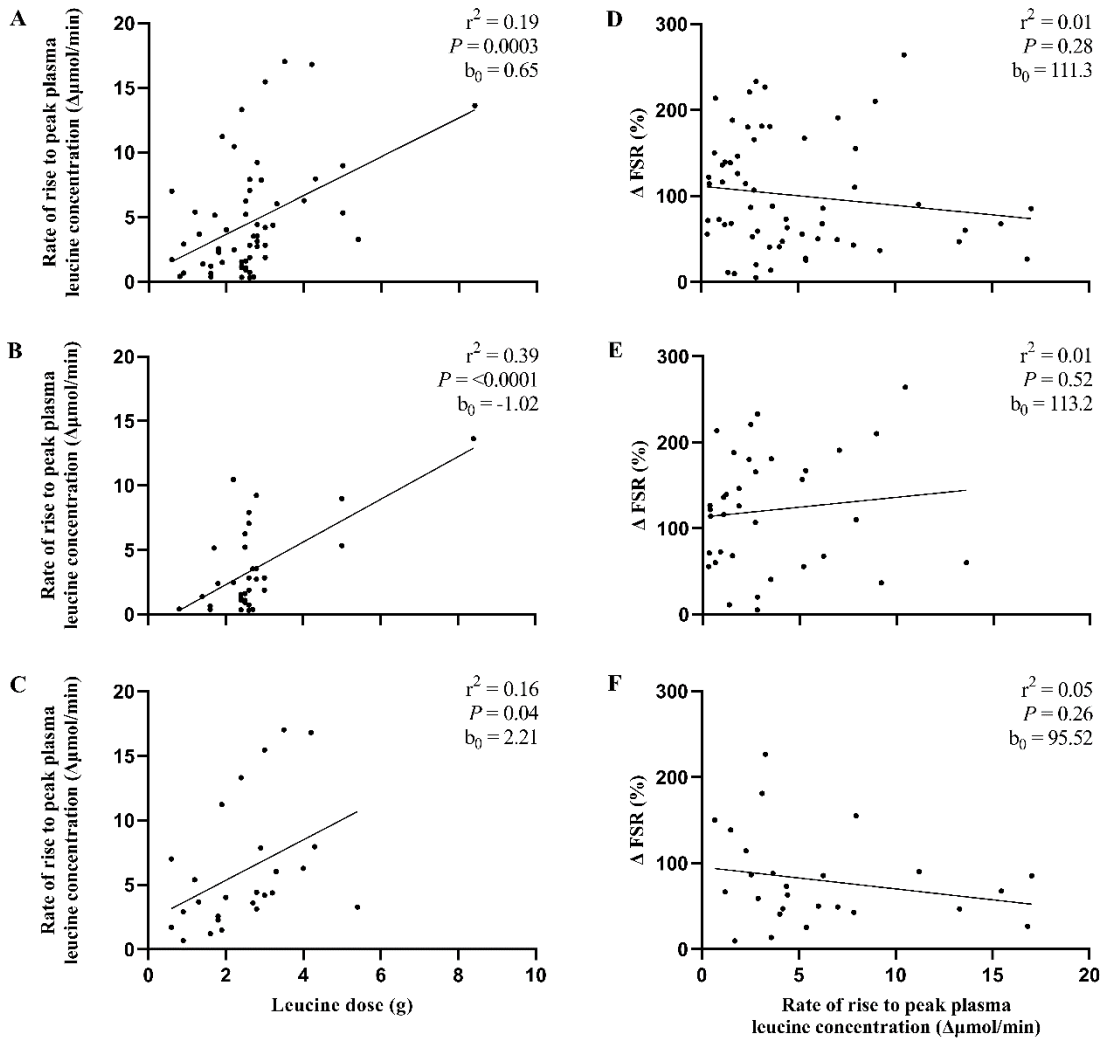

Figure 3.4 *Rate of rise to peak plasma leucine magnitude in response to a leucine dose provided as a bolus protein ingestion within an hour of resistance exercise in all participants (61 study arms)* ***(A),*** *young participants (35 study arms; 19-29 years) (**B**) and older participants (26 study arms; 57-74years) (**C**). Delta change (postprandial post-exercise increase) in muscle protein synthesis rates (MPS), expressed as fractional synthetic rate (FSR) in relation to rate of rise to peak plasma leucine magnitude in all participants* ***(D),*** *young participants (**E**) and older participants (**F**). Rate of rise to peak plasma leucine magnitude determined from the highest plasma leucine concentration minus basal plasma leucine concentration divided by time in minutes to peak concentration. Data were analysed by linear regression; coefficient of determination (r^2),* P *value and y-intercept (b_0) are presented.*

3.3.9 Rate of rise to peak plasma leucine magnitude

When considering the whole data set (**Figure 3.4A**), there was a relationship between leucine dose and rate of rise to peak plasma leucine magnitude (r^2 = 0.19, *P* = 0.0003, b_0 = 0.65). When split for age this relationship between leucine dose and rate of rise to peak plasma leucine concentration was present for both younger (**Figure 3.4B**; r^2 = 0.39, *P* = <0.0001, b_0 = -1.02) and older adults (**Figure 3.4C**; r^2 = 0.16, *P* = 0.04, b_0 = 2.21). However, correlations were not observed between peak plasma leucine concentrations and delta change in postprandial, post-exercise MPS across the entire dataset (**Figure 3.4D**; r^2 = 0.01, *P* = 0.28, b_0 = 111.3) nor when split into younger and older adults (**Figure 3.4E**; r^2 = 0.01, *P* = 0.52, b_0 = 113.2, **Figure 3.4F**; r^2 = 0.05, *P* = 0.26, b_0 = 95.52 for younger and older adults, respectively).

3.3.10 Total postprandial plasma leucine availability

When considering the entire data set (**Figure 3.5Figure 3.4A**), the leucine dose showed a relationship to plasma leucine iAUC (r^2 = 0.58, *P* = <0.0001, b_0 = -5701). With a significant relationship observed between leucine dose and plasma leucine iAUC for both younger (**Figure 3.5B**; r^2 = 0.74, *P* = <0.0001, b_0 = -10850) and older (**Figure 3.5C**; r^2 = 0.65, *P* = <0.0001, b_0 = -1939) adults. However, plasma leucine iAUC showed no relationship to delta change in postprandial, post-exercise exercise MPS rates when considering the whole data set (**Figure 3.5D;** r^2 = 0.02, *P* = 0.27, b_0 = 124.6) and this relationship was not altered when split for age; plasma leucine iAUC showed no relationship to delta change in post-exercise MPS rates amongst the younger (**Figure 3.5E**; r^2 = 0.002, *P* = 0.78, b_0 = 131.7) or older (**Figure 3.5F**; r^2 = 0.01, *P* = 0.65, b_0 = 96.62) adults.

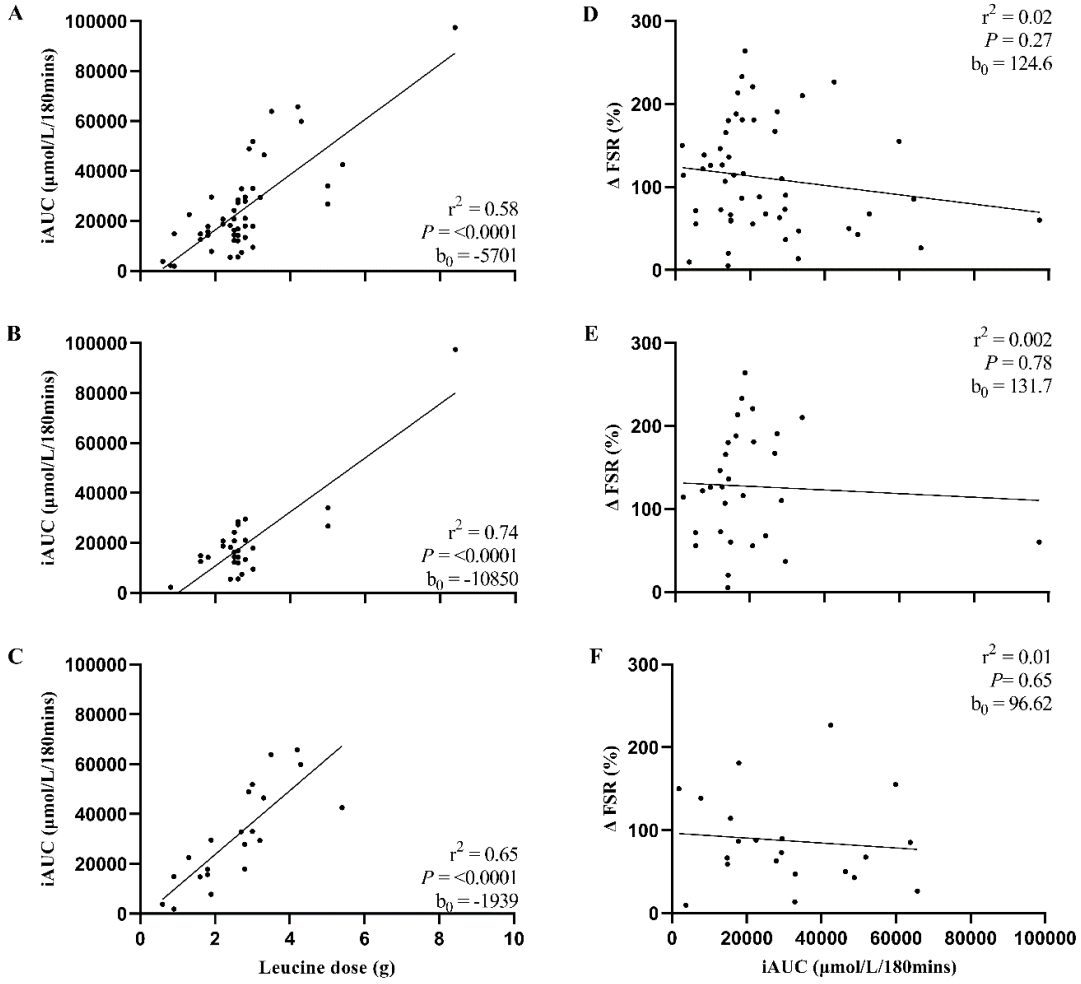

Figure 3.5 *Total postprandial plasma leucine availability, represented as incremental area under the curve over 180minutes, in response to a leucine dose provided as a bolus protein ingestion within an hour of resistance exercise in all participants (61 study arms)* ***(A),*** *young participants (35 study arms; 19-29 years) (**B**) and older participants (26 study arms; 57-74years) (**c**). Delta change (postprandial post-exercise increase) in muscle protein synthesis rates (MPS), expressed as fractional synthetic rate (FSR) in relation to total postprandial leucine availability in all participants (**D**),* ***young participants (E) and older participants (F).*** *Data were analysed by linear regression; coefficient of determination (r^2),* P *value and y- intercept (b_0) are presented.*

3.4 Discussion

3.4.1 Principal findings

In the present quantitative systematic review we provide a detailed examination of the physiological regulation of post-exercise MPS rates by leucine ingested within dietary protein in younger and older adults. We first quantified the predictive capacity of leucine dose *per se* on the magnitude of post-exercise MPS rates. We then sequentially examined the relationships between ingested leucine dose and various aspects of its postprandial post-exercise availability within the circulation. Finally, we assessed the ability of those aspects of postprandial plasma leucine availability to predict post-exercise MPS rates. We report several novel findings that further our understanding of the leucine threshold and trigger concepts. First, ingested leucine dose *per se* is associated with the magnitude of the postprandial post-exercise MPS response, but this relationship exists only in older adults, over both the early and entire measurement periods (**Figure 3.2C**). Second, largely irrespective of age, ingested leucine dose is highly predictive of the peak magnitude, rate of rise, and total availability of plasma leucine concentrations during the postprandial post-exercise period. Finally, when examining these discreet aspects of postprandial post-exercise plasma leucine variables in this reductionist manner, no single variable possessed any association with the magnitude of postprandial post-exercise MPS rates in either young or older adults.

3.4.2 Leucine threshold concept

What is generally referred to as the ‘leucine threshold’ hypothesis, posits a simple dose-response relationship between total leucine ingested and the postprandial post-exercise MPS response, plateauing at around ~2.5 g [168]. This is aligned

with various applied sports nutrition recommendations to ingest a protein meal containing at least 2 to 3 g leucine in close temporal proximity to exercise to maximise the post-exercise muscle anabolic response [165, 305-309]. Our present data do not fully support this concept. There was no correlation between ingested leucine dose and the post-exercise MPS response over a 6 h period (**Figure 3.2**) in the largest cohort of young individuals studied to date. Indeed, the lines for both the early and entire phase of post-exercise MPS intercept (b_0) at around 100%, which could already be maximal (**Figure 3.2B**). Some [171, 289, 297], but not all [214, 296] studies demonstrate an increase in MPS with additional leucine over resistance exercise alone, which raises the question of whether leucine increases MPS over and above the stimulus of resistance exercise in younger individuals at all. Of course, there could be a dose response effect on supressing MPB and, therefore, hypertrophy, but this has not been investigated to date. This also highlights the difficulty in providing precise prescriptions based on leucine alone, especially given the array of differences across subjects and exercise protocols. In contrast, there was a strong dose-response correlation between ingested leucine dose and post-exercise MPS rates in older adults, with overall lower increases observed compared to young. Indeed, in comparison to younger adults where b_0 was around 100%, MPS did not increase to 100% (i.e. double) at all over 2 h, or until around 3-4 g of leucine was ingested during the entire postprandial phase. This is in line with recent similar investigations into the regulation of MPS by leucine in older individuals [166] and likely reflects age-related alterations in digestion and absorption kinetics [310, 311], splanchnic extraction [312, 313], perfusion [314] and/or a reduction in sensitivity (and/or delay in response) of muscle to the anabolic properties of dietary protein (all encompassed within the term ‘anabolic

resistance';[82, 162, 270]). In line, older adults showed a slower rate of rise to peak, greater variability of peak magnitude and an overall 'rightward shift' that was lower, particularly in the early phase, indicating a greater and faster leucine response is required for an equivalent rise in MPS. To a certain extent, this shift to the right could explain the significant correlation in the older adults only, given this provides a greater spread of the data. Irrespective, our findings extend the concept of anabolic resistance to imply that the anabolic sensitivity to leucine becomes of more relevance in terms of governing post-exercise postprandial muscle protein synthesis rates in senescent muscle. However, the linear nature of the relationship between leucine dose and post-exercise MPS rates in older individuals, and lack of an obvious breakpoint, do not reveal a plateau or 'threshold', unlike previous studies that only compare two or three doses [161, 168, 269].

3.4.3 Leucine trigger concept

The utility of comparing leucine dose to post-exercise MPS responses does not account for the multitude of mediating physiological factors that could mechanistically modulate this relationship. Attempts have been made to link the two, generally encompassed within the umbrella term 'leucine trigger' hypothesis [168, 217, 305]. We show that leucine dose strongly predicts various postprandial candidate 'triggers', such as peak plasma leucine magnitude (**Figure 3.3**; [160, 174, 275, 315]), the rate of rise to peak plasma leucine magnitude (**Figure 3.4**; [166, 213]) and total postprandial plasma leucine availability (iAUC; **Figure 3.5**, [316]). However, when comparing these variables against postprandial MPS rates, no relationships were observed in the entire cohort, nor when younger and older adults were considered separately. This is surprising given the observed

association between leucine dose and post-exercise MPS rates, as well as the prevailing wider narrative within the literature where a clear manipulation of postprandial leucinemic variables *per se* and an association with the consequent muscle anabolic response are seen. West et al. [275] reported that a more rapid delivery of leucine to the circulation following bolus whey ingestion translated to greater MPS rates compared with the same quantity consumed in a pulse fashion. Similarly, by comparing ingestion of pre-hydrolyzed casein with intact casein, Pennings et al. [174] showed greater leucinemia conferred a more potent MPS response in older adults.

The lack of any observed associations, within this systematic review, may be explained by 'noise' in the data being too great to pin down one single plasma variable, whereas the leucine dose represented a composite of the total protein dose and all postprandial leucinemic factors thereby revealing the relationship. However, once other variables are introduced, such as comparing different protein sources [214, 231, 291, 304], isolated *vs* whole foods [176, 178, 216], meal ingestion [201, 279] or co-ingestion with other macronutrients [317-320], the relationship is far less clear. We [98, 202, 300] and others [176, 178, 291] have observed a dissociation between circulating leucine concentrations and MPS in a series of recent studies, specifically involving whole food approaches. For example, a 25% greater MPS response was observed with ingestion of skim milk vs. beef despite a significantly lower plasma leucine concentration [178]. In line, a recent systematic review concluded that the leucine trigger hypothesis was predictive of subsequent MPS responses only if protein isolates were consumed on their own [217]. This may also explain why we only observed a relationship between leucine threshold and MPS in older individuals, where all the studies to date have involved protein isolates.

Collectively, therefore, it appears that postprandial plasma leucine responses as the prime determinant of the post-exercise MPS response may be of most relevance when all other factors remain the same and thus leucine availability is limiting. Once other factors are introduced the influence of leucine diminishes and other regulatory candidates and limiting factors (e.g. total protein dose, other signalling or substrate limiting amino acids, other macro/micronutrients, hormonal/incretin/neural, etc.) must be considered. However, the total protein dose provided did not modulate delta change MPS (**Appendix Figure 9.1**), and as such any observed relationships between leucine and delta change MPS did not appear to be primarily driven simply by a greater dose of leucine also being associated with a larger dose of total protein. It is important to note that true plasma leucine kinetics, involving multiple pool modelling of exogenous and endogenous leucine rates of appearance and disappearance, as well intramuscular transport, incorporation, oxidation and efflux, are rarely measured. One might hypothesise that a protein sources 'other factors' aforementioned could speed the rate of disappearance of leucine into muscle tissue for a greater intracellular stimulatory effect on MPS, whilst also lowering peak magnitude, rate of rise and/or total postprandial availability of plasma leucine. Indeed, there has been much debate as to where a potential leucine 'sensor' may reside [106], with an intracellular sensor now considered most likely [321]. Therefore, using plasma leucine variables (only) as proxy markers for MPS triggers may not be an effective tool. We and others may be neglecting key variables such as changes (independently from plasma concentrations) in muscle leucine uptake, intracellular leucine concentration and intramuscular leucine incorporation into polypeptide chains.

3.4.4 Conclusions and limitations

This systematic review collated all studies which have provided a single bolus of protein within one hour of a single bout of resistance exercise and measured the subsequent MPS response. Whilst there is a clear dose-response of ingested leucine with post-exercise MPS rates in older individuals, our data do not identify a precise leucine *threshold,* as no evident plateau was identified, and a maximal MPS response appears to be achievable in young individuals with protein ingestion *per se* irrespective of leucine content. Moreover, we report that the post-exercise postprandial MPS response cannot be predicted from any single plasma leucine variable and, therefore, we cannot confirm the existence (or at least primacy) of a specific physiological leucine *trigger*. As such, our results indicate that both leucine dose and plasma leucine concentrations only explain part of the variability in post-exercise postprandial MPS responses. Given our data are somewhat at odds with *in vitro* findings and some individual studies, we leave open several possibilities that our conclusions may be obfuscated by: lack of data across more diverse leucine doses (most studies provided 2-2.5 g leucine) or corrected to total body/lean mass; few reports involving true postprandial leucine kinetics; lack of intramuscular leucine measurements; altered and/or additional regulation by (as yet unidentified) other macro/micro-nutrients; and the availability of other amino acids required as signal and /or substrate for sustaining optimal MPS rates. Nevertheless, this review has again highlighted anabolic resistance in older individuals, and the importance of study design with older individuals needing to encompass a longer postprandial period to ensure that the whole MPS response is captured.

4 Nutritionally targeting post eccentric exercise recovery

'A society grows great when old men (and women) plant trees who's shade they know they will never sit in.'

Thank you, Grandma and Grandad, for planting the scientific seed.

This chapter is a pooled data set from two clinical trials undertaken within the Nutritional Physiology research group. Data collection and analysis for chapter 4 was performed by Kiera Wilkinson, Amy Booth, George Pavis and Tom Jameson.

4.1 Introduction

As outlined in **Chapter 1**, eccentric exercise has been used as a model to induce skeletal muscle damage and it is well established that when strenuous and unaccustomed, this forcible lengthening of the muscle, induces a loss in muscle function, increases muscle soreness [18, 76] and alters muscle protein metabolism [144]. This loss of muscle function appears immediately and may continue for days post unaccustomed eccentric exercise, taking ~2-7days to recover [33, 34, 49, 56, 59, 60, 186, 322]. The successful repair, remodelling and recovery of muscle force generating capacity is likely to be dependent upon the anabolic ability of the muscle to synthesis new functional skeletal muscle proteins [78]. The anabolic effect of exercise can be enhanced by the exogenous provision of amino acids [93].

Without additional macronutrients, plasma essential amino acids are the main drivers for the postprandial elevation in myofibrillar protein synthesis (MPS) [313]. This availability of plasma amino acids over the early (0-2h) [178] and entire (0-6h) [98, 170] postprandial period, provide substrate to maintain MPS and prevent a net negative protein balance [87]. A dose response relationship has been demonstrated to exist between MPS and dose of protein, with a linear increase up to ~20g protein [168, 169]. This maximised response has also been seen with 20-30g protein, providing 2-3g leucine [164], and similarly with essential amino acids only [93] or with the addition of essential amino acids added to an inadequate dose of protein [214]. Leucine has been characterised as having a molecular role in stimulating the mTORC1/P70S6K signalling pathway associated with muscle protein synthesis [103, 107, 171, 273]. This has led to recommendations of ingesting 20-25g protein which provides 2-3g of leucine in close temporal proximity to exercise to maximise the post-exercise muscle

anabolic response [165, 305-309]. However, within **chapter 3**, we have demonstrated that there is no dose response relationship between postprandial post exercise muscle protein synthesis and the dose of leucine or subsequent postprandial leucinemia variables. Such that amongst young (18-35y) healthy adults a maximised MPS response may be initiated with a resistance exercise stimulus alone. This highlights the difficulty in providing precise prescriptions based on leucine alone and in relation to recovery from strenuous damaging exercise.

A maximised anabolic response following exercise is thought to be the mechanism that alleviates muscle soreness and accelerates recovery of muscle function following strenuous damaging exercise [59, 61, 77, 78, 127, 186, 188]. However, the effect of protein ingestion on functional recovery post eccentric exercise is equivocal with studies reporting positive outcomes of accelerated recovery [59, 77, 78, 127, 188] and others reporting no differences between the exogenous provision of protein or carbohydrate placebo supplementation [60, 61, 193]. The utilisation of branched chain amino acids has been demonstrated to reduce muscle soreness following eccentric exercise, however this did not occur alongside improvements in muscle function [49], suggesting that all essential amino acids may be required for the recovery of muscle function. The consumption of 30g whey protein isolate daily following eccentric exercise accelerated recovery of muscle strength by 72h compared to a carbohydrate placebo [188]. Similarly the consumption of milk protein blends has been shown to accelerate recovery of muscle function over 48h following eccentric exercise [77, 127]. The observed disparities in the outcomes of supplementation following eccentric exercise could be attributed to the variability in exercise and study protocols as show in **Table 1.1,** such that the degree of muscle damage and

therefore the repair and remodelling will vary. Further disparities could be due to variability in amino acid provision from participant's habitual diet. To control this, strict dietary control can be employed as well as utilising a unilateral within subject contralateral control leg exercise model throughout the intervention phase to ensure that the primary factor influencing post eccentric exercise recovery is the targeted nutritional intervention.

A single bout of resistance exercise with the addition of exogenous protein source increases MPS responses by more than ~100% as demonstrated in **Chapter 3**. Eccentric contractions induce a greater mixed and myofibrillar muscle protein synthetic response than concentric contractions [88, 144]. However the mechanistic insight into muscle protein synthetic responses following eccentric exercise and how this influences post eccentric exercise recovery is sparse. We seek to understand if muscle protein metabolism dictates functional recovery following eccentric exercise alongside the provision of ~20g protein and ~2g leucine, utilising a deuterated water dosing protocol to allow the free-living integrated measurement of muscle protein synthesis over days of recovery.

The effect of eccentric exercise and the subsequent loss in muscle function has been investigated in predominantly a young healthy male population (**Table 1.1** [31, 33, 34, 49, 56, 59-61, 77, 127, 188, 193, 322]). However there may be considerable differences between the acute response to exercise between males and females [323]. Females experience large cyclic fluctuations in endogenous hormones throughout the three main phases of the menstrual cycle; follicular, ovulation and luteal [324]. How these hormones affect muscle performance and function is still yet to be fully elucidated [325]. The current evidence base may not be representative of how female muscle recovers and responds to protein

nutrition following eccentric exercise. Alongside suggestions of no differences between males and females muscle protein synthesis rates at rest or post exercise thereby suggesting no effect of endogenous circulating hormones [326-329]. In addition to addressing the effect of protein on MPS following recovery from eccentric exercise, we seek to determine if there are differences between males and females. Further to implicate whether the investigation of both males and females should be considered for subsequent chapters of this thesis.

We draw upon studies completed within the Nutritional Physiology research group, that have undergone a voluntary bout of unilateral eccentric exercise. As well as utilising deuterated water consumption to allow the measurement of free living integrative MPS to be able to capture the time course of recovery and understand if changes in recovery are dictated by protein and leucine provision and the subsequent anabolic response. We hypothesised that with the post exercise provision of protein designed to nutritionally target exercise recovery this would result in improved recovery of muscle function following eccentric exercise in both males and females and increases in myofibrillar protein synthesis, with similar functional and metabolic responses between males and females.

4.2 Methods

Using high force eccentric exercise protocols, two studies consisting of 5 study groups have assessed the effects of protein nutrition on the recovery of muscle function. Within both these studies the same eccentric exercise protocol, muscle function testing and muscle soreness visual analogue scales were utilised. The data presented in this chapter is a collation of these two studies completed within the Nutritional Physiology Research Unit, University of Exeter. The studies were approved by the University of Exeter's Sport and Health Sciences Research

Ethics Committee (Ref. No. 161026/B/06, 190703/B/01) and both registered as a clinical trial with ClinicalTrials.gov (NCT02980900, NCT04156386). All individuals had provided written consent prior to volunteering to take part in the study. Once data was collated from respective studies, data was split into male and female participants who either consumed a protein beverage or a placebo beverage post exercise. All procedures are explained briefly below; see Pavis, Jameson [78] and within **Chapter 2** for a detailed overview.

4.2.1 Participants

Forty-seven healthy recreationally active participants; defined as participating in sporting activities >2h·wk^{-1} but not following a structured exercise training programme, specifically no more than 2 resistance training sessions per week, were recruited across the two separate clinical trials. Exclusion criteria were: 1) diagnosed metabolic or cardiovascular impairment; 2) self-reported habitual protein intake<0.8g·kg^{-1}·day^{-1}; 3) musculoskeletal injury that may impair exercise performance; and/or 4) engagement in systematic resistance training within 6 months of participation. Eumenorrheic females with regular cycles, not using an oral contraceptive, took part in the experimental period between days 0-14 of a regular menstrual cycle. If this was not possible days 7-9 of the study protocol fell between days 7-14 of the menstrual cycle (i.e. mid follicular phase). Those who were using oral contraceptives were studied at any time.

Table 4.1 *Participant characteristics*

	Female PLC (n= 9)	Male PLC (n =9)	Female PRO (n=14)	Male PRO (n=15)	
Age, y	22.4 ±1.1	21.1 ±0.4	23 ±1.4	21 ±0.6	
Height, cm	169.1 ±2.2 #	176.2 ±1.5 #	166.3 ±1.7*	177.7 ±1.7	* *P* <0.05
Body mass, kg	66.7 ±4.2	73.3 ±4.4	61.3 ±3.1*	73.4 ±2.1	* *P* <0.05
BMI, $kg.m^{-2}$	23.2 ±1.4	23.5 ±1.2	21.9 ±0.7	23.1 ±0.5	
Eccentric work, $J.kgBM^{-1}$	506.4 ±68.2	645.3 ±73.8	531.5 ±38.9	618.3 ±45.3	
Baseline function (CON leg), J	2069.7 ±260.5*	2855.7 ±224.0 #	1754.4 ±117.5*	2520.1 ±159.5	* # *P* <0.05
Baseline function (ECC leg), J	2193.8 ±290.2*	2889.8 ±150.3 #	1806.0 ±85.7*	2561.8 ±165.3	* # *P* <0.05
Energy, $kcal.day^{-1}$	2286.2 +112.2*	2837.1 ±119.6 #	2252.2 ±89.3*	2871.6 ±58.9 #	* *P* <0.001
Carbohydrate, $g.day^{-1}$	337.7 ±12.0*	410.03 ±10.6 #	309.6 ±9.2*	396.4 ±6.7 #	* *P* <0.0001
Fat, $g.day^{-1}$	71.6 ±5.2	90.9 ±6.8 #	64.9 ±4.6*	87.1 ±3.6	* *P* <0.001
Protein, $g.day^{-1}$	78.1 ±4.0 ^	88.8 ±5.5 ^	95.6 ±3.3* #	111.9 ±2.4* #	^ * # *P* < 0.005
Protein, $g.kgBM.day^{-1}$	1.2 ±0.01 ^	1.2 ±0.0 ^	1.6 ±0.0* #	1.5 ±0.03* #	^ * # *P* <0.0001

Values are presented as means ±SEM. PRO group consumed a daily protein supplement for 7 days prior to and 7 days following a bout of 300 maximal eccentric contractions of the knee extensors. ECC leg is the leg that performed the eccentric contractions. CON leg is the contralateral control leg that did not perform the eccentric contractions. * indicates differences between males and females within the same condition, # indicates differences between males and females in different supplemental conditions, ^ indicates differences between conditions for the same sex

4.2.2 General study design

Following screening and acceptance into the study, all participants completed a familiarisation visit, at least 48h prior to commencing the study. Participants were familiarised to the muscle soreness measurement, exercise testing equipment and protocols. The familiarisation consisted of the muscle function test and 5 submaximal repetitions of the eccentric exercise, to ensure we did not induce a repeated bout effect.

All participants completed a 14-day study period whereby they underwent strict dietary control and daily supplementation. Participants were randomly assigned to one of two double blind parallel groups, consisting of either a daily protein (PRO, *n* =29) or placebo (PLC, *n* =18) drink (*see Diet and Nutritional Intervention*). The experimental and controlled diet period commenced, with baseline measures of muscle function and soreness, followed by the consumption of the experimental drink.

On day 7 of the experimental protocol, a unilateral bout of maximal eccentric muscle contractions of the knee extensors was performed using a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Shirley, New York, USA). The eccentrically exercised leg (ECC) was randomly counter balanced for leg dominance with the contralateral leg acting as the within participant control (CON). Muscle soreness measured using a 100mm visual analogue scale (VAS) and total isokinetic work over 30 maximal concentric contractions of the knee extensors was measured in each leg separately every 24h (up to 168h) following eccentric exercise.

4.2.3 Eccentric contraction protocol

On day 7 of the 14 day protocol, all participants performed 300 (10 set of 30 repetitions) voluntary maximal, unilateral isokinetic eccentric contractions of the knee extensors. Each contraction was performed with an angular velocity of $60 \cdot s^{1}$over an 80° range of motion. Each set was separated by 120-s rest. Participants were instructed to resist the movement maximally through the full range of motion and provided verbal encouragement throughout.

4.2.4 Muscle function and soreness testing

Muscle function and soreness were determined as previously described [78, 186]. After an overnight fast participants arrived at the laboratory at baseline and 24-168h following eccentric exercise for measures of muscle soreness and total isokinetic work. Participants were asked to rise from a seated position and rate general lower body muscle soreness. Measured using a 100mm VAS scale with 'no soreness' and worst soreness possible' anchored at 0mm and 100mm, respectively. Muscle function was determined by the bilateral assessment of total isokinetic work, measured as area under the torque time curve after 30 maximal, concentric, isokinetic contractions of the knee extensors, performed at $75°\cdot sec^{-1}$ through an 80° range of motion equidistant from voluntary maximal knee extension and flexion, and were preceded by a 5-repetition submaximal warm-up. Four additional sets of 30 maximal concentric contractions were completed after the functional assessment. Each set was separated with 60 s rest. Verbal encouragement was provided throughout the exercise.

4.2.5 Diet and Nutritional Intervention

All participants underwent strict dietary control for 14 days to maintain energy balance and provide $1.2g\cdot kg$ body $mass^{-1}\cdot day^{-1}$ protein. Energy requirements were based on the Henry equation [253] multiplied by an activity factor of 1.6. Participants were informed of the importance of adhering to the diet. All food was individually weighed, packaged and provided with instructions of how to prepare each meal. Compliance with the controlled diet was assessed by participants returning completed daily food logs, empty food containers and daily communication. No other foods or energy containing beverages were permitted throughout the study but, water was permitted ad libitum.

The nutritional interventions provided were a commercially available post exercise beverages (Beachbody Performance Recover: Beachbody LLC, Santa Monica, CA, **Table 2.1**) containing either 21.9g total protein from whey (2.4g leucine, 480mg tart cherry extract), 24.8g total protein from pea (2.2g leucine, 650mg pomegranate extract) or 20g total protein from a blend of whey, pea and casein (2g leucine, 650mg pomegranate extract). Participants in the PLC group received an isocaloric maltodextrin placebo drink containing 31g carbohydrate. The experimental beverages were consumed once daily, immediately post exercise during the laboratory visits or at home during the initial dietary control phase.

4.2.6 Deuterated water loading protocol

The deuterated water dosing protocol consisted of 1 loading day (day 4) and 3 maintenance days to achieve and maintain 0.6% body water deuterium enrichment. On day 4, participants arrived at the laboratory overnight fasted (~08:00am) and consumed 6 mL·kg body mass^{-1} of deuterated water (70 atom%: Cambridge Isotope Laboratories Inc, Tewksbury, MA,USA) across 8 doses pulsed over 1.5 h intervals. Following the first dose, participants were provided with their daily supplement and breakfast. Participants remained in the laboratory until after their fourth dose was consumed and then returned home to consume the remaining four doses. For the maintenance days (days 7,8 and 9), participants consumed a daily dose of 0.54 mL·kg body mass^{-1} 70% deuterated water upon waking.

4.2.7 Muscle biopsy collection

Muscle biopsies were collected from the mid region of the *vastus lateralis* (~15cm above the patella) with a modified Bergström needle under local anaesthesia (2%

lidocaine). All biopsy samples were immediately freed from any visible blood, adipose or connective tissue, and were frozen immediately in liquid nitrogen and stored at -80°C until subsequent analysis. Muscle tissue collection was 24, 48 h and 72 h (See *methods section 2.5.2*) [78] after the bout of maximal unilateral eccentric exercise. At every time point, bilateral muscle biopsies were taken from the ECC leg and the CON leg. Successive biopsies were collected from a different incision, approximately 2cm proximal to the previous incision.

4.2.8 Plasma and muscle analysis

Body water deuterium enrichments (from plasma samples) and myofibrillar bound [^{2}H]alanine enrichments, extracted from ~30-50mg of wet weight muscle tissues were analysed at the University of Texas Medical Branch using isotope ratio mass spectrometry (IRMS) and gas chromatography mass spectrometry (GC-MS) respectively, as previously published [78] and described in **Methods Section *2.5.3*** and ***2.5.6***.

4.2.9 Calculations

Muscle function measured as isokinetic work in the eccentrically exercised leg, was corrected to the contralateral control leg (%CON) prior to statistical analysis.

Daily MPS rates were calculated [expressed as fractional synthesis rates (FSR)] based on the incorporation of the mean body water deuterium enrichment over the 3-d intervention as a precursor pool into myofibrillar bound proteins. FSR was calculated using the standard precursor-product method and expressed as daily rates as follows:

$$FSR\ (\%{\cdot}d^{-1}) = \frac{\Delta E\mathrm{p}}{3.7\ \mathrm{x}\ Ep\ \mathrm{x}\ t}\ x\ 100$$

where Δ*E*p is the increment in [^{2}H]alanine enrichment in myofibrillar protein between two biopsies (1-2/3*d*), $E_{precursor}$ (E*p*) is the average body water deuterium enrichment between two biopsies corrected by a factor of 3.7 based upon the deuterium labelling of alanine during *de novo* synthesis, and *t* indicates the tracer incorporation time between two muscle biopsies.

4.2.10 Statistical analysis

All data is presented as mean ± standard error of the mean (SEM). All statistical analysis was performed on GraphPad Prism version 9.5.1 (GraphPad Prism). Statistical significance was set at $P < 0.05$. Differences in subject characteristics, dietary intake, baseline muscle function and eccentric work done were analysed using a one factor analysis of variance (ANOVA).

A repeated measured three-factor ANOVA (PLC compared with PRO, FEMALE compared with MALE and TIME) was used to. Alongside a further three-factor ANOVA for myoFSR data (PLC vs PRO, Female vs Male and CON leg vs ECC leg). Repeated 2-factor ANOVA (PLC vs PRO and TIME) was used to compare muscle function and muscle soreness, separately. When significant main effects were found, Sidak post hoc tests were applied to locate individual differences.

4.3 Results

4.3.1 Participant characteristics

Recreationally active participants (*n* = 47) were recruited and randomised to consume either maltodextrin placebo (PLC) or a form of protein nutrition (PRO). For the purpose of this analysis, results are split by sex (male *n* = 24, female *n* = 22). There were no differences between male PLC and PRO participants for age or BMI. Differences between males and females and between conditions for body mass and height are presented in **Table 4.1.**

Average daily energy and macronutrient intake during the 14-day controlled diet after accounting for compliance and supplementation is presented in **Table 4.1.** Statistical differences in energy and macronutrient intake seen across males and females and between conditions are presented in **Table 4.1**.

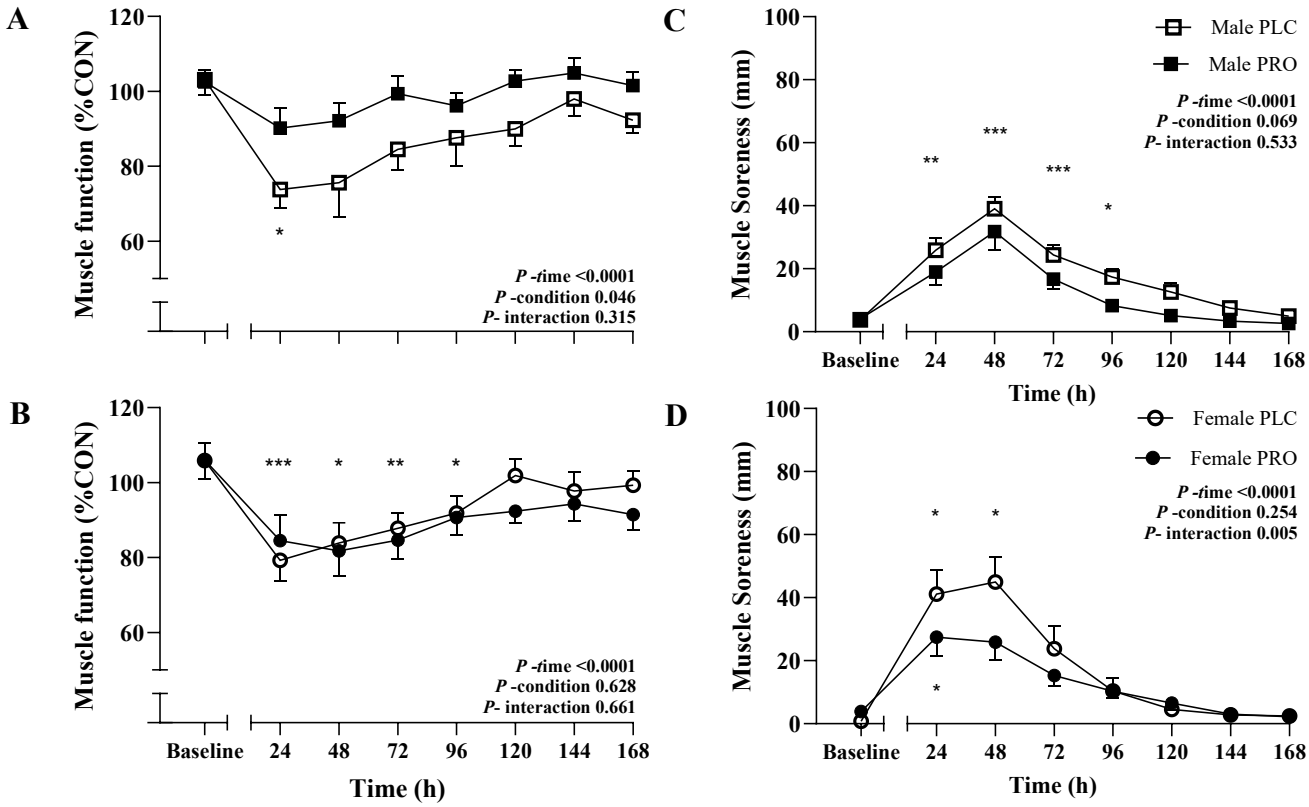

Figure 4.1 *Muscle function and muscle soreness, following a bout of 300 maximal eccentric exercise. Male (**A and C**) and female (**B and C**) data is presented separately. Baseline values were measured 7 days prior to the bout of eccentric exercise. Knee extensor isokinetic work, measured over 30 maximal unilateral concentric contractions, expressed relative to the control leg. Muscle soreness, measured on a 100mm visual analogue scale (VAS). Both were recorded every 24 h following a bout of 300 maximal unilateral eccentric contractions of the knee extensors. Data is from two clinical trials and is split into males and females, consuming either a maltodextrin placebo (PLC) or a protein supplement (PRO) daily for 7 days prior and 7 days following the bout of eccentric exercise. Data are presented as means with error bars representing standard error. Statistical analysis was performed with two factor ANOVAs. Time points significantly*

different to baseline (main effect) are presented with * P <*0.05,* ** P < *0.01,* *** P < *0.001.*

4.3.2 Muscle function

Baseline muscle function, measured as isokinetic work done over 30 maximal concentric contractions, were different between males and females in the both the CON leg (*P* = 0.0013) and ECC leg (*P* = 0.0006). Female PRO CON leg was significantly different to male PRO and PLC CON leg. Female PLC CON leg was significantly different to male PLC CON leg. Female PRO ECC leg was significantly different to male PLC leg. Female PLC ECC leg was significantly different to male PLC ECC leg.

Isokinetic work, expressed relative to the control leg, was measured every 24 h after 300 maximal unilateral eccentric contractions. Male and female muscle function is shown in **Figure 4.1A** and **B**. Separate analysis was performed on males and females to determine the influence of protein supplementation on both sexes separately.

Total isokinetic work decreased from baseline in males following eccentric exercise after 24h (by 34 ±15%, *P* =0.039), in the PLC condition and returned to baseline by 48h. However PRO supported recovery of muscle function with post hoc tests indicating no significant differences compared to baseline (condition effect *P* =0.046, post hoc differences between PLC and PRO *P* > 0.05).

Total isokinetic work decreased from baseline in females following eccentric exercise after 24h (by 27 ±17% in PLC and 29 ±20% in PRO, *P* =0.0003), 48h (by 23 ±23% in PLC and 29 ±25% in PRO, *P* =0.021), 72h (by 23 ±17% in PLC and 27 ±21% in PRO, *P* =0.011). The change in female muscle function was not

different between PLC and PRO conditions (**Figure 4.1B**, condition effect *P* = 0.628).

4.3.3 Muscle soreness

Muscle soreness in the male cohort increased from baseline up to 48h (PLC: 39.0 ±11.75 and PRO: 31.82 ±21.81mm) and was higher than baseline until 96h post eccentric exercise (main time effect; *P* <0.0001, **Figure 4.1C**). Muscle soreness was no different to baseline values at 120 h post eccentric exercise (*P* = 0.528). Muscle soreness in the PLC condition was consistently and trended to be greater than the PRO condition (main condition effect; *P* = 0.06).

Eccentric exercise significantly increased muscle soreness from baseline values in females and to a different extent between conditions (**Figure 4.1D**, time x condition interaction; *P* =0.005). PLC condition muscle soreness was greater than baseline at 24 h (41.11 ±22.68mm, *P* = 0.019) and 48 h (44.94 ±24.38mm, *P* = 0.016) post eccentric exercise. The female PRO condition showed significant differences from baseline only until 24 h (27.42 ±21.47mm, *P* = 0.030) post eccentric exercise.

4.3.4 Myofibrillar protein synthesis

Daily myoFSR 1-3 days after eccentric exercise, was unaffected by sex, condition, or prior eccentric exercise (leg x condition x sex interaction effect; *P* = 0.872). There were no differences between the CON leg (1.64 ±0.34%·d^{-1} and 4.06 ±1.09%·d^{-1}, *P* =0.184) or ECC leg (2.22 ±0.46%·$d^{-1}$and 3.93 ±0.98%·d^{-1}, *P* =0.419) for female PLC or PRO conditions, respectively. There were no differences between the CON leg (2.04 ±0.24%·d^{-1} and 2.69 ±0.46%·d^{-1}, *P* =0.287) or ECC leg (2.51 ±0.26%·d^{-1} and 2.67 ±0.42%·d^{-1}, *P* =0.783) between male PLC or PRO conditions, respectively. There was a trend for a greater daily

myoFSR in the PRO condition compared to the PLC condition (condition effect, *P* = 0.068).

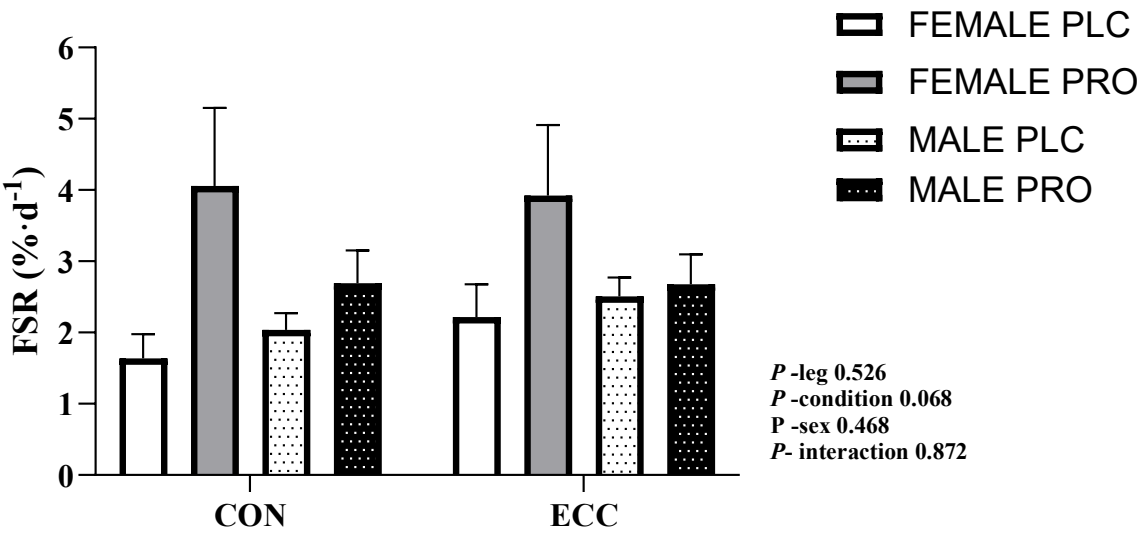

Figure 4.2 *Myofibrillar protein synthesis measured utilising a deuterated water loading technique to allow the measurement of free living integrative fractional synthetic rate (FSR) over 24- 72hours (2 days) post eccentric exercise. Muscle biopsy tissue collection occurred 24hours (1 day) and 48/72 hours (2/3 days) post exercise from both the eccentrically exercise leg (ECC; 300 maximal unilateral eccentric contractions) and the contralateral control leg (CON). Data is from two clinical trials and is split into males and females, consuming either a maltodextrin placebo (PLC) or a protein supplement (PRO) daily throughout a 14-day controlled diet period. FSR was calculated using the product precursor equation and is expressed as a percentage per day. Data is presented as means with error bars representing standard error.*

4.4 Discussion

This study aimed to characterise the time course of recovery when providing 20-25g protein with ~2g leucine, designed to nutritionally target functional recovery and to determine the importance of MPS in this recovery process. Alongside determining if females and males both respond to a protein nutritional intervention and recovery from eccentric exercise. These pooled studies were not originally powered to detect statistical differences between sexes, however

due the same study design, a larger sample size and this being a developing research area in regard to female physiology this study was warranted. As expected, eccentric exercise, across both males and females, induced a transient decline in muscle function and an increase in muscle soreness following high volume maximal eccentric exercise. Protein supplementation accelerated recovery in males within the participant cohort, with function not dropping below baseline in males over any time point. However females experienced a reduction in muscle function at 24h and this was not recovered until 4 days post eccentric exercise recovery, highlighting a differing response to protein nutrition between sexes. The loss in muscle function also coincided with peaks in muscle soreness with an earlier rise in females at 24 h and higher peak at 48 h post exercise in females compared to males (F: 44.94 ±24.38mm, M: 39.0 ±11.76mm). This study demonstrates that males and females recover over a different time course, with protein nutrition designed to nutritionally target post exercise recovery accelerating recovery in this male cohort only. The accelerated recovery rate with protein nutrition as opposed to placebo supplementation may be attributed to the trend for an enhanced anabolic response associated with protein intake following eccentric exercise over 24-72h recovery.

By utilising a unilateral exercise model, we take out the potential confounding variable of males having greater initial strength and producing greater total eccentric and concentric work, due to all data being expressed as a percentage of the individuals control leg or normalised to body mass (**Table 4.1**, **Figure 4.1**). The eccentric exercise protocol induced moderate muscle damage [32], represented by a 20-40% reduction in muscle force generating capacity, in both

males and females. Within the male cohort, muscle function dropped below baseline for 24h following eccentric exercise eliciting a 34% drop in muscle function compared to baseline, whereas protein supplementation protected the ability to generate force with no statistical differences to baseline over the recovery period. This drop in muscle function amongst recreationally active males is consistent with previous eccentric exercise models in the acute recovery period post exercise and the ability for a protein supplement to accelerate the rate of functional recovery [34, 60, 61, 188, 193].

The extended recovery time frame in females suggests that this eccentric exercise stimulus was more novel and induced more metabolic and morphological disruption. Previous comparisons have shown that the force loss and rate of strength recovery are similar between males and females, such that the force lost immediately may be greater [330, 331] but the rate of recovery and extent of muscle soreness are no different between males and females [332]. However within this female cohort, recovery took 4 days with or without the influence of protein. This recovery time frame amongst the female cohort was undertaken during the early follicular phase, when endogenous sex hormones are at their lowest, with evidence to show a greater loss in muscle strength following eccentric cycling exercise [333] and higher pain perception [334], compared to findings in the mid luteal phase, where endogenous sex hormones are highest, which has been associated with a smaller loss in muscle function and soreness [335]. Within the present study design, the phase of the menstrual cycle was based on calendar based counting and the onset of menses rather than gold standard method of serum hormone analysis [336], such that future studies should endeavour to accurately determine phases of the menstrual cycle

to facilitate a deeper understanding into if hormonal changes influence exercise recovery and protein turnover.

The post exercise protein supplementation providing 20-25g protein and ~2g leucine effectively accelerated the recovery of muscle function in recreationally active males compared to an isocaloric placebo by 24h. To deduce why there may be potential differences between supplementation conditions, we compared muscle protein synthetic responses in the days following exercise where the greatest reductions in muscle function are seen following eccentric exercise and whereby recovery was accelerated when consuming a protein supplement. Across both collated studies, a deuterated water dosing protocol was utilised to allow the free-living integrated measurement of muscle protein synthesis over days of recovery. We further add to the current evidence that there are no differences between males and females muscle protein synthesis rates post resistance exercise [326-329], and here we present no difference between the eccentrically exercised (ECC) and the contralateral control (CON) legs, but highlight a trend for the protein condition daily myofibrillar protein synthesis rates to be higher than the placebo condition over a 24-72h period post eccentric exercise. Whilst the MPS values did not reach significance, this demonstrates that elevated MPS rates may underpin an element of post eccentric exercise recovery.

Within the male group, recovery had already occurred by 24 h which supports the conclusions drawn by Pavis, Jameson [78], that the recovery of muscle function may not dictated by elevated rates of muscle protein synthesis. Alternatively, the maximised and synergistic influence of exercise plus protein may have occurred earlier in the recovery time frame. We know that maximised MPS responses are

achievable in young healthy adults with exercise and protein ingestion *per se* irrespective of leucine content (**Chapter 3**) and we show that a nutritional intervention (20-25g protein and 2g leucine) can expedite muscle recovery and soreness following high volume eccentric exercise within the initial 24-48 h. Suggesting that the initial 24 and 48 hours represent a critical period during which the predominant recovery of muscle function occurs. The integrated assessment of MPS across a 24–72 h recovery timeframe may have encompassed this heightened MPS response, but it does not manifest explicit distinctions between conditions, likely attributed to the potential elevation in MPS rates during this earlier temporal window, which will be explored in **Chapter 5**. However we demonstrate that elevated MPS rates may underpin accelerated recovery with the provision of exogenous amino acids providing substrate at the site of damage for increased myofibrillar remodelling.

We provide evidence that nutritionally targeting post eccentric exercise recovery with the provision of 20-25g protein and ~2g leucine can accelerate the recovery of muscle function amongst recreationally active males and reduce the incidence of muscle soreness in both males and females. This response may be underpinned by a trend for elevated rates of MPS induced by the additional provision of protein nutrition such that this increased muscle protein turnover at the site of injury can accelerate muscle functional recovery. However we suggest that there is an earlier time frame where elevated rates of MPS may be evident which supports the accelerated functional recovery. Subsequent chapters within this thesis will investigate these earlier time periods and whether this influences functional recovery when consuming a plant-based protein intervention.

5 The effect of a plant-based protein source on the recovery of muscle function and myofibrillar protein synthesis rates following eccentric exercise

'You can't control the wind, but you can adjust your sails.'

The data presented in this chapter is part of a larger clinical trial. Data collection and analysis was undertaken by Kiera Wilkinson and Amy Booth. Placebo group data within this chapter has been included in a PhD thesis submitted to the University of Exeter by Amy Booth to address a separate research question.

5.1 Introduction

The ability of the muscle to remodel and recover following muscle damage is crucial for subsequent exercise bouts and the regulation of muscle function and mass. We [78, 133, 186, 252] and others [31, 337, 338] have utilised a high-volume eccentric exercise model, whereby recreationally active participants performed 300 maximal eccentric contractions, to experimentally induce a loss in force generating capacity. With the greatest decrements of 15-40% in isometric or isokinetic maximal voluntary force (**Chapter 4**, [339]) being over the initial 48h following exercise [49, 56, 60, 127, 190] and taking up to 7 days for muscle function to be fully restored [322]. This coincides with peaks in muscle soreness [67] and increases in markers of cytokine mediated inflammation [186] which become prevalent within 48 hours. Losses of force generating capacity have been diminished [78] or reduced [77, 187] with the consumption of high quality protein sources such as milk protein [77], whey protein [59] or a whey protein blend [78]. Antioxidants [338] and polyphenols [340, 341] also have the capacity to accelerate recovery from damaging exercise. Pavis, Jameson [78], demonstrated that the consumption of a protein blend (20g) with 650 mg pomegranate extract, providing 2g leucine, accelerated recovery of muscle function by 5 days compared to a carbohydrate placebo. It has been demonstrated that protein [49] and protein polyphenol supplementation [78] reduces the extent and time course of post exercise muscle soreness, with marked attenuation at 48h following eccentric exercise. This increase in muscle soreness has been associated with the disruption to contractile structures and cell walls, subsequent cytokine release and infiltration of leukocytes into the muscle [342]. This infiltration of leukocytes has been suggested to lead to oedematous swelling and subsequent osmotic pressure, activating nociceptors and eliciting the feeling of soreness [71]. The

relationship between muscle swelling and muscle soreness has not been investigated following eccentric exercise and the consumption of a protein polyphenol intervention used to attenuate inflammation and muscle protein breakdown.

It has been suggested that muscle damage is vital for the remodelling process and recovery of muscle function [35, 40, 41, 78, 186, 343, 344], implicating that the elevated MPS response is a critical process for repair and recovery and that the greater availability of amino acids would support and enhance this response. However, recent findings suggest that the improvement in muscle function following strenuous resistance exercise [195] and eccentric exercise [78] are not underpinned by greater MPS after feeding with essential amino acids or protein compared to a carbohydrate placebo. Findings from **Chapter 4**, demonstrate a trend for an elevated MPS rate over 24-72h, however muscle function had returned to baseline by 24h, suggesting that MPS rates could have been greater earlier in the acute hours following exercise as such preventing further aberrant damage and thus preventing a decline in muscle function (explored in **Chapter 6**) or as discussed in **Chapter 4**, the time frame between 24-72h may not have captured the changes in the acute days following the damage stimulus. The majority of recovery of muscle function occurred in the initial 24h following eccentric exercise suggesting that there is a time period over 24 to 48h post eccentric exercise whereby nutritional intervention would be beneficial [78]. We therefore wanted to capture the integrated MPS response over the acute days, utilising a deuterated water dosing protocol, to understand whether MPS rates underpin recovery and if a plant-based protein supplement can support this response.

Plant based protein sources have increased in popularity due to the growing consumer demand for healthy, ethical and sustainable products [345], however have been considered less anabolic following resistance exercise [160, 173]. This has been attributed to the differing essential amino acid profiles (EAA) and subsequent patterns of aminoacidemia [206, 207, 246], specifically the lower leucine content not 'triggering' MPS maximally. However, **Chapter 3** demonstrated that amongst younger adults (18-40y) a maximal MPS response appears to be achievable with protein ingestion *per se* irrespective of leucine content and source of protein, following resistance exercise. To our knowledge, the potential of a plant-based protein to accelerate recovery following eccentric exercise is yet to be determined, specifically pea protein which is now a widely consumed protein supplement, due to being readily available, widely grown and an inexpensive pulse crop [235]. Within **Chapter 4** we demonstrated that providing a ~20g dose of protein alongside ~2g leucine is effective for recovery following damaging exercise in recreationally active males and females. We therefore utilised a plant-derived protein source (*Pisum Sativum*) combining a high leucine content and no apparent amino acid deficiencies to nutritionally target post eccentric exercise muscle recovery, such that it would elevate MPS.

To our knowledge, the association between myofibrillar protein synthetic response, the recovery of muscle function, soreness and swelling following eccentric exercise, alongside the consumption of a plant-based protein supplement has not been directly investigated. We hypothesised that nutritionally targeting amino acid availability, through the consumption of a plant-based protein supplement, providing 25g protein and 2.2g leucine, post eccentric exercise would accelerate recovery of muscle function, suppress muscle

soreness and swelling, alongside increasing myofibrillar protein synthesis in the acute days following eccentric exercise.

5.2 Methods

5.2.1 Participant characteristics

Nineteen young, healthy recreationally active males (*n* = 8) and females (*n* = 11) volunteered to take part in the present study (age: 22 ±0.7 y, height: 171.1 ±2.0cm, body mass: 64.4 ±2.1kg, BMI 21.9 ±0.5kg·m^{-2}). Participant characteristics are displayed in **Table 5.1**. All individuals provided written consent after being provided verbal and written explanation of the experimental procedures. The study was approved by the Sport and Health Sciences ethics committee (Ref No. 190703-B-01) and is registered at ClinicalTrials.Gov (ID: NCT04156386).

Table 5.1 *Participant characteristics*

	PLC (*n=9, 6F, 3M*)	VGP (*n=10, 5F, 5M*)
Age (y)	22 ±1.1	22 ±0.9
Height (kg)	169.2 ±2.5	172.8 ±3.1
Weight (cm)	62.1 ±3.1	66.4 ±2.9
BMI (kg·m^{-2})	21.7 ±0.8	22.2 ±0.6
ECC total work (J)	32634 ±5293	37918 ±3422
Baseline work CON leg (J)	2101 ±311	2013 ±171
Baseline ECC leg (J)	2151 ±287	2095 ±158

Values represented as mean ±SEM. PLC: daily maltodextrin placebo supplementation, VGP: daily vegan pea protein supplementation; BMI: body mass index. ECC total work represents the total volume of eccentric work performed during the 300 eccentric contractions performed in the randomised dominant or non-dominant leg. Baseline work represents the total volume of work done in the muscle function test in the ECC: eccentrically exercised leg and the CON: control leg.

5.2.2 Study protocol

Details of all experimental procedures are presented in **Chapter 2.** A schematic overview of the experimental protocol is shown in **Figure 5.1.** All participants completed a 14-day study period whereby they underwent strict dietary control and daily supplementation (**Section 2.4.3**). Participants were randomly assigned to one of two parallel groups, consisting of either a daily vegan pea protein (VGP) or placebo (PLC) supplement. **Table 5.2** summarises the daily energy and macronutrient intake.

All participants were familiarised with the exercise equipment and protocols at least 48 hours prior to commencing the experimental protocol. Baseline visit (day 1) and subsequent days 8- 14 participants arrived overnight fasted at 0800 h for a blood sample, measurements of muscle soreness and muscle function were taken. Baseline or day 7 (dependent on availability) were used for MRI scanning followed by subsequent scans on day 8 and 9. At ~1000 h on day 7, the eccentric exercise was performed in one leg (ECC), randomly assigned, and counterbalanced for leg dominance. The contralateral leg was the control (CON). Muscle function was then assessed every 24h following the eccentric exercise bout with an additional four sets of 30 isokinetic knee extensor contractions to maximise the post- exercise muscle protein synthetic response, followed by the consumption of the post exercise supplement. To determine muscle protein synthesis rates post-eccentric exercise and supplementation, participants underwent a deuterated water dosing protocol to determine myofibrillar protein synthesis rates in both the ECC leg and CON leg. This allowed participants to continue with ‘free-living’ during the 14-day experimental protocol, whilst undergoing strict dietary and exercise control. The dosing protocol commenced with a loading day and continued with daily maintenance doses on days 7-9.

Participants underwent bilateral *m. vastus lateralis* muscle biopsies, prior to eccentric exercise (day 7) and the subsequent 24 (day 8) and 48 h (day 9) after eccentric exercise to determine rates of myofibrillar protein synthesis.

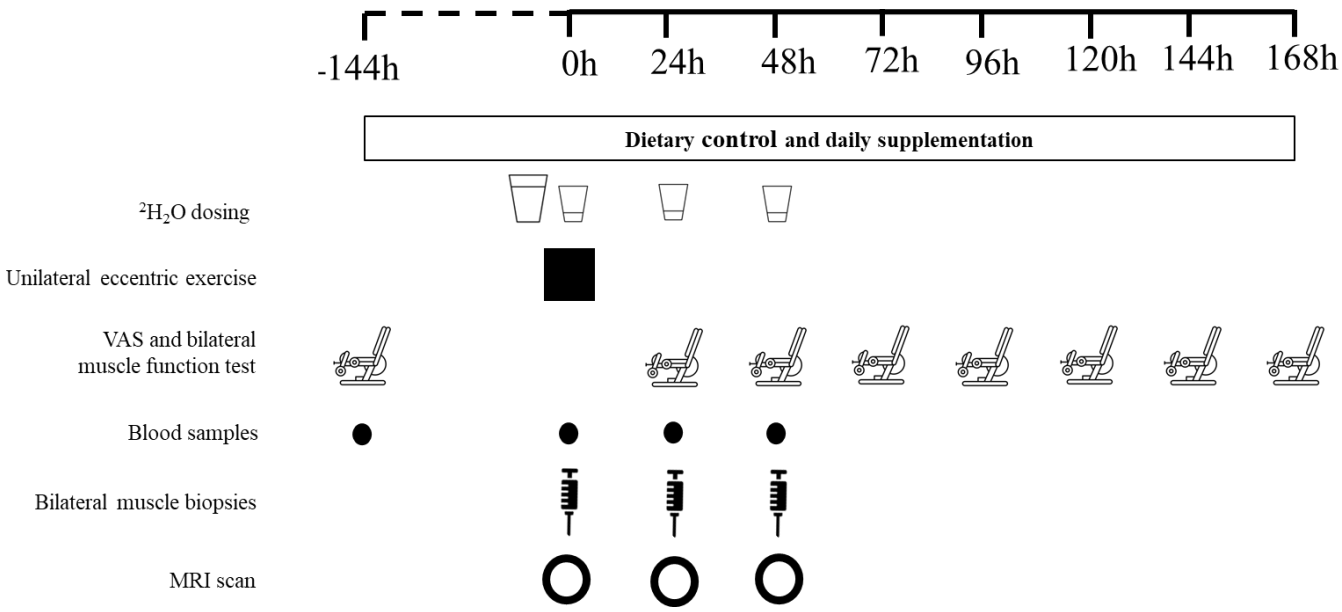

Figure 5.1 *Schematic representation of the experimental protocol. A 14-day controlled diet study period and daily supplementation of either a vegan pea protein supplement (VGP) or maltodextrin placebo (PLC) was employed throughout the study. Oral consumption of 70% ^2H_2O began at ~0800 on the day 4, with 8 x 0.75 mL·kg^{-1} doses consumed every 1.5h and maintained thereafter with daily doses of 0.54 mL·kg^{-1}. Bilateral muscle function and soreness was measured at baseline (-144 h pre-eccentric exercise) and every 24 h following a bout of eccentric exercise. Unilateral eccentric exercise (10 sets x 30 repetitions) was performed after 7 days of dietary control (t = 0 h). Blood sampling was performed pre-eccentric exercise (t = 0 h) and subsequent 24 and 48 h following eccentric exercise bout. Bilateral muscle biopsies were obtained at 0, 24 and 48 h after eccentric exercise to measure myofibrillar muscle protein synthesis. MRI scans were performed pre-eccentric exercise (t = 0 h), 24 and 48 h following eccentric exercise, in the axial plane to measure whole thigh and quadriceps muscle volume.*

5.2.3 Experimental Drinks

Drinks were provided by the manufacturer (Beachbody LLC, Santa Monica, California, USA) in sachets which contained a single serving of supplement. These sachets were coded to ensure that double blinding was maintained. The VGP supplement was fortified with methionine to raise the content to above the 1.6% protein content recommendations set out by the World Health Organization [206]. The nutritional content of the supplements is displayed in **Table 2.2**, within this experimental chapter supplements PLC and VGP were used.

Table 5.2 *Average daily energy and macronutrient intake consumed during a 14 day controlled diet*

	PLC (*n=9, 6F, 3M*)	VGP (*n=10, 5F, 5M*)
Habitual energy intake ($kJ \cdot day^{-1}$)	8616.3 ±1062.9	8363.3 ±743.3
Habitual protein intake ($g \cdot kgBM^{-1} \cdot day^{-1}$)	1.5 ±0.3	1.3 ±0.1
Controlled diet (energy contribution %)		
Energy intake ($kJ \cdot day^{-1}$)(%)	9382.7 ±467.0	10738.7 ±473.8
Carbohydrate intake ($g \cdot day^{-1}$)(%)	353.9 ±18.7 (60)	345.0 ±16.8(54)
Fat intake ($g \cdot day^{-1}$)(%)	64.1 ±3.3 (24)	70.2 ±3.9 (25)
Protein intake ($g \cdot day^{-1}$)(%)	74.3 ±3.7 (13)	104.9 ±3.5 (17)**
Controlled diet PRO (excluding treatment) ($g \cdot kgBM^{-1} \cdot day^{-1}$)	1.2 ±0.0	1.2 ±0.0
Controlled diet PRO (including treatment) ($g \cdot kgBM^{-1} \cdot day^{-1}$)	1.2 ±0.0	1.6 ±0.0 ***

Values represented as mean ±SEM. PLC; daily maltodextrin placebo supplementation, VGP: daily vegan pea protein supplementation Habitual protein intake analysed from a 3-day food diary. Controlled diet protein intake relative to body mass per day with and without supplementation. (%) percentage contribution to daily energy intakes. ** $P < 0.01$, *** $P < 0.0001$ significantly different to PLC

5.2.4 Blood sample collection

A venous blood sample, from the antecubital vein, via venepuncture, was collected on day 1 from participants when they arrived overnight fasted (~0800h) and on day 7, 8 and 9.

5.2.5 Skeletal muscle biopsy collection

Muscle tissue collection occurred on day 7 (0 h) and subsequently 24 and 48 h after the bout of maximal unilateral eccentric exercise. At every time point, bilateral muscle biopsies were taken from the ECC leg and the CON leg. Successive biopsies were collected from a different incision, approximately 2cm proximal to the previous incision.

5.2.6 Calculations

In line with previous work investigating recovery of muscle function after unaccustomed eccentric contractions, isokinetic work in the eccentrically exercised leg was corrected to the contralateral control leg (%CON) prior to statistical analysis. This was used to reduce intra- and inter- individual variation.

Myofibrillar protein fractional synthesis rates were calculated based on the incorporation of [^{2}H]alanine into myofibrillar protein and mean plasma deuterium enrichment between muscle biopsy timepoints using the following standard precursor-product equation:

$$FSR\ (\%{\cdot}h^{-1}) = \frac{\Delta E\mathrm{p}}{3.7\ \mathrm{x}\ Eprecursor\ \mathrm{x}\ t}\ X\ 100$$

where Δ*E*p is the increment in [^{2}H]alanine enrichment in myofibrillar protein between two (0, 24 or 48 h) biopsies, $E_{\mathrm{precursor}}$ is the average body water

deuterium enrichment between two biopsies corrected by a factor of 3.7 based upon the deuterium labelling of alanine during *de novo* synthesis, and *t* indicates the tracer incorporation time between two muscle biopsies.

5.2.7 Statistical analysis

Differences in subject characteristics, dietary intake and total eccentric isokinetic work between conditions were analysed using unpaired t-tests. A repeated measure 2-factor analysis of variance (ANOVA) was used to analyse (condition x time) differences in muscle function, muscle soreness, muscle volume (%CON) and body water ^{2}H enrichment. A repeated measure 2-factor ANOVA was used to analyse (condition x leg) differences in myofibrillar fractional synthetic rate (FSR). A repeated measure 3-factor ANOVA (leg x condition x time) was used to determine differences in myofibrillar protein bound [^{2}H]alanine enrichment. When a significant main and interaction effect was observed, a Sidak post hoc test was performed to locate individual differences. Calculations were performed using GraphPad Prism 9.5.1. Statistical significance was set at $P < 0.05$. All data are expressed as means ±standard error of the mean (SEM).

5.3 Results

No differences in participants age, body mass, height or BMI were found between groups (all $P > 0.05$, **Table 5.1**). Average daily energy and macronutrient consumption during the 14 day controlled diet, after accounting for compliance, is presented in **Table 5.2**. When accounting for the ingestion of daily supplementation, VGP increased daily protein intake compared with PLC over the 14-day controlled diet period (1.5 v 1.2 $g \cdot kgBM^{-1} \cdot day^{-1}$, respectively, $P < 0.001$). Total work performed during the eccentric exercise bout (10 x 30 repetitions) did not differ between conditions (**Table 5.1**). All data is presented as

PLC (*n* = 9) and VGP (*n* = 10) except for MRI scan analysis (PLC *n* = 5, VGP *n* = 8) due to the availability of the MRI scanner not fitting within the research schedule.

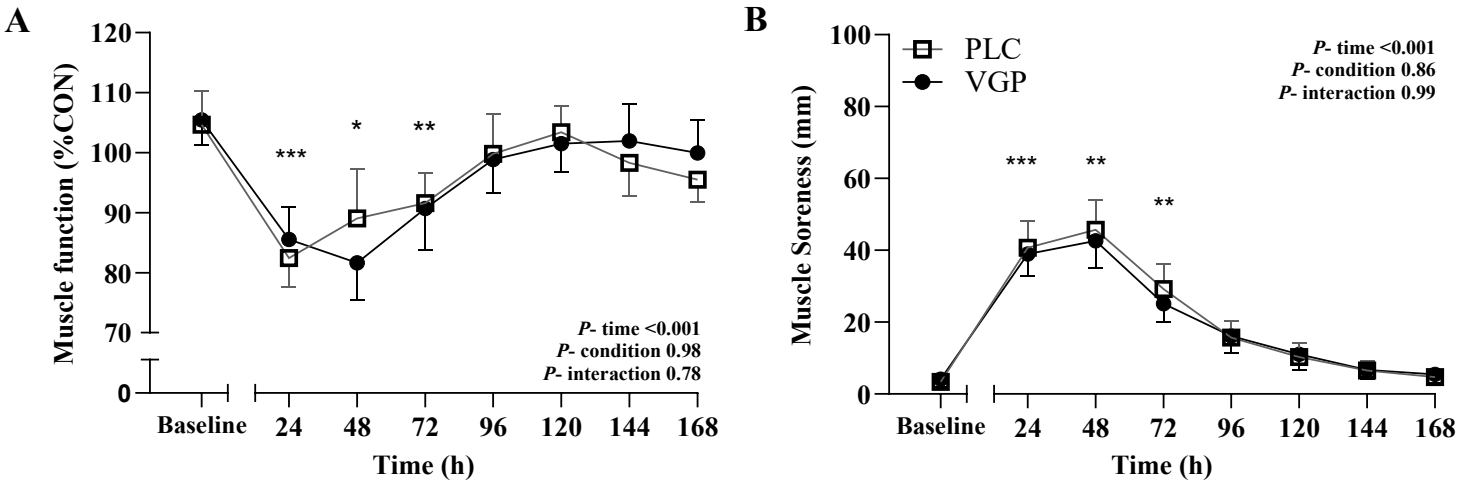

Figure 5.2 *Knee extensor isokinetic work (**A**) expressed relative to the control leg and visual analogue scale muscle soreness (**B**) recorded on a 100mm scale (0mm no soreness and 100mm worst possible soreness), following a bout of 300 maximal eccentric exercise. Baseline values were measured 7 days prior to the bout of eccentric exercise. Post exercise vegan pea protein (VGP;* n = *10, black circles) or a maltodextrin placebo (PLC;* n =*9, white squares) supplement were ingested for 7 days prior and 7 days following the bout of eccentric exercise. Data are presented as means with error bars representing standard error. Statistical analysis was performed with two factor ANOVAs (time x condition). Time points significantly different to baseline (main time effect), post hoc differences to baseline are presented with* * P *<0.05,* ** P < *0.01,* *** P < *0.001.*

5.3.1 Muscle function

Muscle function, measured as total isokinetic work, expressed relative to the control leg (%CON) is shown in **Figure 5.2A.** Baseline muscle function did not differ between PLC and VGP (104.7% and 105.5%CON respectively, post hoc *P* >0.05 vs baseline). After a bout of voluntary unilateral maximal eccentric contractions, muscle function decreased in PLC and VGP at 24 h (-22.22 ±5.10%

and -15.55 ±5.87%CON, respectively, time effect; $P < 0.05$), with ingestion of post exercise supplementation having no effect on this change in muscle function ($P > 0.05$). For both PLC and VGP muscle function remained suppressed until 72 h (91.58 ±5.10% and 90.70 ±6.86%, post hoc $P < 0.05$ vs. baseline) post eccentric exercise, with function returning to baseline values by 96 h for both groups ($P > 0.05$) .

5.3.2 Muscle soreness

Baseline muscle soreness was 3.4 ±1.8mm and 4.1 ±2.2mm in PLC and VGP, respectively (**Figure 5.2B**, $P > 0.05$). Muscle soreness increased from baseline at 24h (post hoc $P < 0.0001$) after a bout of voluntary maximal unilateral eccentric exercise and peaked at 48 h in both PLC and VGP (45.7 ±8.1 and 42.6 ±7.4 mm, respectively; post hoc $P < 0.0001$ vs baseline) and had returned to baseline by 96 h post eccentric exercise ($P = 0.08$). The supplementation of VGP did not have an influence over muscle soreness compared to PLC condition ($P = 0.86$).

5.3.3 Skeletal muscle volume

Skeletal muscle volume is expressed relative to the control leg, for total thigh muscle volume and quadriceps muscle volume (**Error! Reference source not found.**). Thigh muscle volume did not change following eccentric exercise ($P = 0.34$) and there were no differences between conditions ($P = 0.32$). Similarly, there were no differences between quadricep muscles volume following eccentric exercise (time x treatment interaction $P = 0.69$).

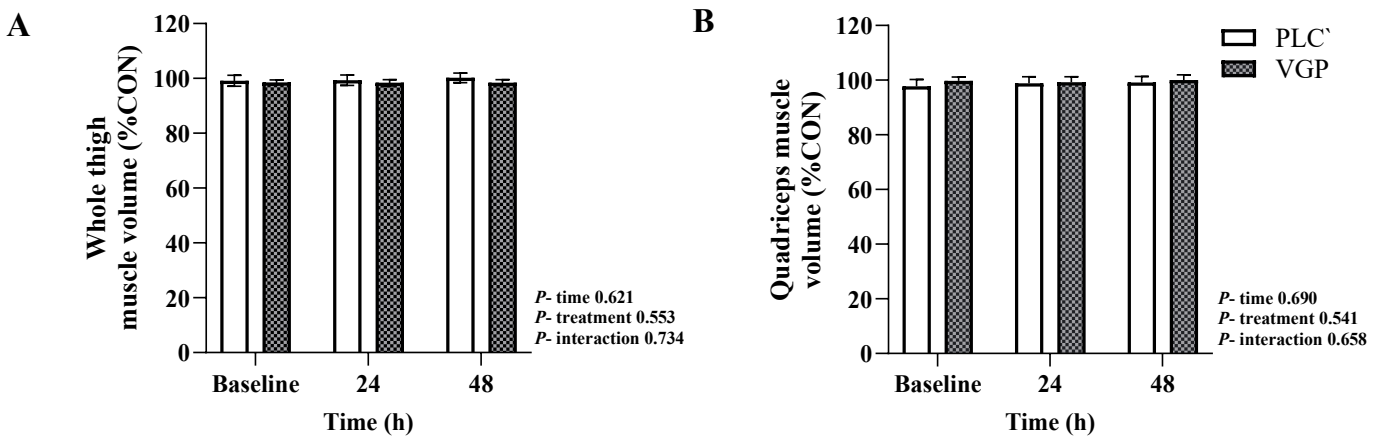

Figure 5.3 *Whole thigh (**A**) and quadriceps (**B**) muscle volume measured using MRI scans at baseline and the subsequent 24 and 48 h following a bout of 300 maximal eccentric exercise. Post exercise supplementation of vegan pea protein (VGP n= 8) and an isocaloric maltodextrin placebo (PLC; n=5, white bars) were consumed daily throughout the duration of the study period. Data is expressed relative to the control leg (%CON) and presented as means with error bars representing standard error. Statistical analysis was performed with two – factor ANOVA.*

5.3.4 Plasma Precursor Enrichment

Body water enrichment, following loading, reached 0.56 ±0.03% in PLC and 0.58 ±0.04% in VGP. Over the 3-day maintenance period, steady state isotopic enrichment was maintained throughout, averaging 0.58 ±0.02% in PLC and 0.54 ±0.02% in VGP. There were no differences between PLC and VGP conditions at any time point (**Figure 5.4A,** *P* >0.05).

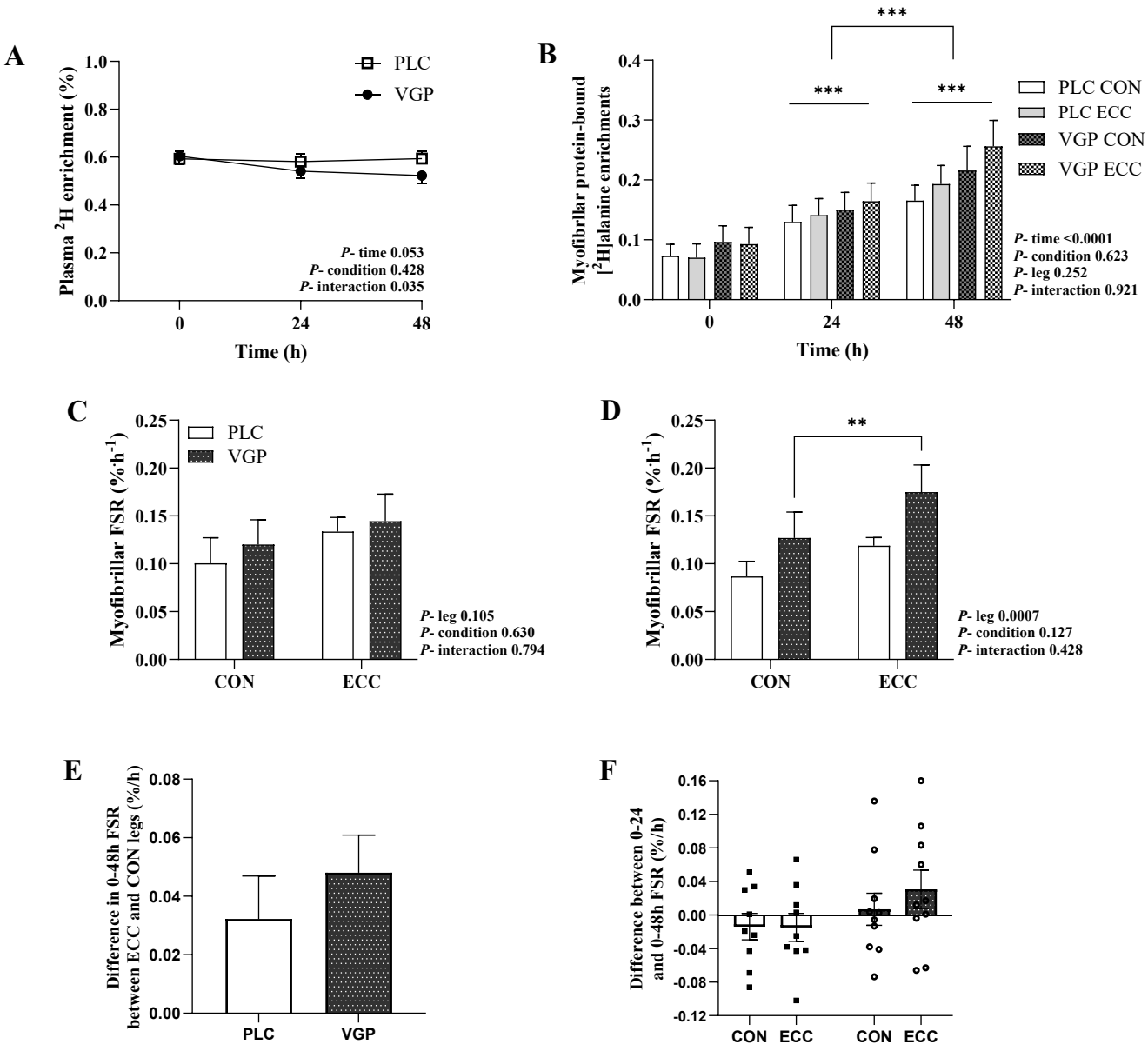

Figure 5.4 *Daily plasma ^{2}H enrichment (%) after oral ^2H_2O loading and maintenance doses. Isotopic equilibrium was maintained from before 300 maximal eccentric contractions and the subsequent 2 days following exercise. Daily plasma ^{2}H enrichment was analysed by 2-way ANOVA (time x condition).* ***B.*** *Enrichment of myofibrillar protein bound [^{2}H]alanine (MPE) from skeletal muscle biopsy samples. Skeletal muscle biopsies were obtained from the ECC (eccentric exercised) leg and CON (contralateral control) leg before (0 h), 24 h and 48 h following a bout of 300 maximal eccentric contractions. Analysed by 3-way ANOVAs (time x condition x leg factors). Main effect of time; *** P <0.0001 different to previous time point.* ***C and D.*** *Myofibrillar protein fractional synthesis*

rate (FSR, expressed as %.h^{-1}) over a 24 h (0-24h, ***C.****) and 48 h (0-48h,* ***D.****) post eccentric exercise, the consumption of post-exercise vegan pea protein (VGP) or maltodextrin placebo (PLC) and the continuation of dietary control providing 1.2g.kgBM·day^{-1}.* ***E.*** *The difference in myofibrillar FSR between the ECC and CON leg over 0-48h.* ***F.*** *The difference between 0-24 and 0-48h cumulative FSR responses. The eccentrically exercised leg is presented as ECC after performing 300 maximal unilateral eccentric contractions and the contralateral control leg is presented as CON. FSR rates were calculated using the product precursor equation. Data are presented as means with error bars representing standard error. Statistical analysis was performed with a two-way ANOVA (leg x condition). Significant main effect of leg (CON vs. ECC) ** P<0.01.*

5.3.5 Myofibrillar protein-bound [^{2}H] alanine enrichment and myofibrillar protein synthesis

Myofibrillar protein-bound [^{2}H] alanine enrichment (**Figure 5.4B**) increased from basal over 24 h and 48 h (main time effect, post hoc *P* <0.001), however these increases were no different between legs (ECC v CON) for both PLC and VGP conditions. Myofibrillar protein-bound [^{2}H] alanine enrichment increased from 24 h to 48 h (post hoc *P* < 0.0001).

Free living postprandial post-exercise myoFSR are displayed in **Figure 5.4C**. During the 48 h recovery period after eccentric exercise myofibrillar FSR was calculated. Over the initial 24 h of recovery (0-24 h), myoFSR was similar between PLC and VGP (main condition effect; *P* >0.05) in both the ECC leg (PLC 0.134 ±0.015, VGP 0.144 ±0.028%·h^{-1}; *P* >0.05) and the CON leg (PLC, 0.101 ±0.027 and VGP, 0.120 ±0.026%·h^{-1}, *P* >0.05). Over the 24 h following the eccentric exercise bout, the ECC leg myoFSR was not different to the contralateral control leg (CON, main leg effect; *P* > 0.05).

Postprandial post-exercise myoFSR over the 48 h post exercise recovery is displayed in **Figure 5.4D.** The VGP ECC leg had a significantly higher 0-48h post-

exercise myoFSR (0.175 ±0.028%·h^{-1}) compared to CON leg (0.127 ±0.027%·h^{-1}, P = 0.004), after both legs had undergone a muscle function test and additional exercise set at 24h, alongside the ECC leg undergoing the eccentric exercise bout. The PLC group showed no differences between legs (CON; 0.087 0.016%·h^{-1}, ECC; 0.119 0.009%·h^{-1}, post hoc P >0.05). Despite a greater myoFSR in the VGP ECC leg, there were no differences in post-exercise myoFSR between conditions (P = 0.127).

5.4 Discussion

The aim of the present study was to characterise the time course of recovery of muscle functional ability, soreness and swelling and determine the muscle protein synthetic response after maximal voluntary eccentric exercise in recreationally active adults, alongside the consumption of a plant-based protein supplement consumed daily over 7 days of recovery. We utilised a deuterated water dosing protocol to measure integrated muscle protein synthesis (MPS) and implored strict dietary control to ensure that any differences observed could be attributed to the additional plant-based protein supplement. Contrary to our hypothesis, pea protein supplementation, consumed immediately post exercise and daily during the recovery period, did not assist in the recovery of muscle function or soreness compared to a maltodextrin placebo in a healthy recreationally active male and females. However, we observed a greater integrated MPS response over the 48h period post exercise when consuming pea protein in the ECC leg compared to the CON leg, indicating an upregulated anabolic response which accelerated the rate of recovery beyond this time frame.

As highlighted in **Chapters 3** and **4** by providing a sufficient protein dose (24.8g) [169], leucine content (2.2g) [159] and with the addition of fortifying with

methionine to allow the pea protein to be classified as a whole protein source (0.5g, 2% of total protein [206]), we expected to see a similar effect on functional recovery following strenuous exercise, as we did in previously published work utilising a protein blend (whey, casein and pea) supplement providing 20g protein and 2g of leucine [78] and within **Chapter 4**. Utilising the same high volume maximal eccentric exercise amongst recreationally active participants and in agreement with previous work [78, 186], we induced a transient decline in muscle function, with the greatest reductions in muscle function being ~25% at 48h. However muscle function remained below baseline values until 96h post exercise in both plant-based protein (VGP) and placebo (PLC) conditions (**Figure 5.2A**). In comparison to functional ability being restored within 24h following the ingestion of the animal derived protein blend [78]. This drop in muscle functional ability coincided with delayed onset of muscle soreness (**Figure 5.2B**), peaking at 48 h post exercise, which is consistent with previous findings of soreness ratings being 40% greater at 48h following damaging exercise and declining from then onwards [67, 78]. However branched chain amino acids have been shown to significantly reduce muscle soreness at 48h by 10% and an animal-derived protein and polyphenol supplement ameliorated muscle soreness by ~20% compared to carbohydrate placebos. Whereas here we demonstrate that a plant-based protein supplementation had no effect on attenuating muscle soreness. The peaks in soreness could not be attributed to inflammatory associated oedema with no changes in muscle volume over the acute days when soreness was peaking, and muscle function was lowest (**Figure 5.3**). We anticipated an increase in muscular volume due to potential structural damage to muscle fibre components, which could lead to the leakage and release of proteins, resulting in

elevated osmotic pressure and subsequent oedema. However, the extent of muscle disruption might not have been severe enough to induce these changes.

The ability of an animal-derived protein supplement compared to a plant-based protein supplement to improve functional recovery and ameliorate muscle soreness may suggest that the type of protein utilised may be of importance. Milk derived peptides have been demonstrated to have anti-inflammatory properties [346], which could attenuate leukocyte upregulation and infiltration. With milk consumption altering the release of inflammatory and damage markers such as IL-6 and CK release [185]. These markers are not affected by the ingestion of carbohydrate following eccentric exercise [347] which is suggestive that peptides found in milk/ animal derived protein may have protective anti-inflammatory properties preventing further aberrant damage or can accelerate the recovery and remodelling process such that muscle function is not hindered.

A further experimental aim of this clinical trial was to investigate the acute hours post eccentric exercise, whereby we investigated functional and substrate metabolism changes 3 hours post exercise and consumption of the supplemental drink, which is outlined in **Chapter 6**. This involved an additional muscle function test, consisting of 30 maximal concentric isokinetic contractions of the knee extensors. This exercise performed in the acute hours following eccentric exercise could increase protein turnover at the site of damage which could help promote recovery of muscle function within 24h (explored in **Chapter 6**). Muscle function at 24h was reduced by 27.05 ±5.13% and 23.24 ±4.37% compared to baseline in PLC and VGP conditions, respectively. These reductions are in line with those seen in Jameson, Islam [252] (~20%), which utilised the same eccentric exercise protocol and performed an additional bout of concentric

exercise 3 and 6h following eccentric exercise. In comparison, greater reductions in muscle function (~35-40%) at 24 h were reported following the same eccentric exercise protocol without the additional function testing (exercise stimuli) 3h following eccentric exercise [78]. This exercise induced increase in protein turnover may be a rationale as to why muscle function decline was not as severe over the acute days following eccentric exercise compared to other studies utilising the same protocol [78]. However, despite these differences, we have presented findings that demonstrate pea protein supplementation does not accelerate the recovery process following eccentric exercise and this suggests that there are other influencing factors which assist in accelerated recovery from eccentric exercise.

Findings from **chapter 4** demonstrate that there was a trend for a greater MPS response with the provision of protein supplementation designed to nutritionally target post eccentric exercise recovery however, the time frame between 24-72h may not have captured the changes in the acute days following the damage stimulus, with the majority of recovery of muscle function occurring in the initial 24h following eccentric exercise suggesting that there is a time period over 24 to 48h post eccentric exercise whereby nutritional intervention would be beneficial. Over the initial 24h post exercise, we show similar rates between the control (CON) and eccentrically (ECC) exercised legs (0.131%/h) across both conditions. We are confident that the lack of effect of protein supplementation is not due to amino acid availability being limited. Across both PLC and VGP conditions, they were provided with sufficient protein intake (1.2 and 1.6 $g \cdot kgBM^{-1} \cdot day^{-1}$, respectively) for muscle protein anabolism [276]. Further, **Chapter 3** demonstrated that the provision of leucine *per se* is sufficient to maximise the anabolic response in young individuals following resistance exercise and that this

provision of 20-25g protein providing ~2g leucine explored in **Chapter 4** can effectively improve recovery following eccentric exercise. The large anabolic responses in both ECC and CON legs across both conditions are greater than have previously been reported in acute (hours) postprandial post resistance exercise tracer studies (0.06-0.1%·h^{-1}, **Chapter 3 Supplementary Information**) [160, 202] and in comparison to pea protein supplementation consumed post whole body resistance exercise (0.087%·h^{-1}, [245]). Alongside being greater than those utilising a deuterated water dosing protocol, over the acute days following resistance exercise (0-48h: ~0.08%·h^{-1}) [115] and the acute days following eccentric exercise plus the consumption of a protein polyphenol supplement (24-72h; ~0.09%·h^{-1}) [78]. Similarly high responses have been seen 24h following eccentric stepping action exercise (~ >0.12%·h^{-1}) in recreationally active males following [348]. Over the initial 0-24h presented here and over 24-72h presented by Pavis, Jameson [78], without any differences between conditions or between exercised legs, it appears that the myofibrillar protein synthetic response does not underpin any differences in functional recovery.

Over the initial 24hours of post eccentric exercise recovery, we suggest that eccentric exercise elicits an acceleration in muscle protein breakdown to provide endogenous amino acids as precursors to support the elevated MPS rates required for remodelling and functional recovery [87, 88, 93, 349]. Endogenous amino acids being drivers for large anabolic responses has been demonstrated within severe burns patients whereby a large breakdown of muscle tissue alongside an increase in rates of MPS demonstrate the disposal of amino acids into the muscle cell such that they can be recycled to support wound healing and skeletal muscle remodelling [119]. The extent of MPB following eccentric exercise would be far lower than a critically ill state however following strenuous exercise

gene expression markers associated with MPB pathways have been elevated [78, 186] as well as rates of MPB have been shown to be elevated 24h following eccentric exercise [88], such that with additional exogenous amino acids this may have no further effect on elevating MPS rates because the delivery of amino acids from breakdown may be prioritised. However, this assumption ignores the option of exogenous amino acids providing substrate to spare skeletal muscle from being broken down alongside downregulating inflammatory and proteolytic pathways to prevent further aberrant damage. It is yet to be determined whether muscle protein breakdown is a primary or secondary response to eccentric exercise such as whether MPB is upregulated to provide precursors for *de novo* muscle protein synthesis or whether it is a by-product of mechanical damage and inflammatory induced pathways that induce proteolysis is not clear. We have been unable to explore muscle protein breakdown rates utilising deuterated water and implore that muscle protein turnover is measured in future studies looking into recovery following damaging exercise to be able to determine whether an exogenous provision of amino acids is beneficial to spare skeletal muscle from muscle protein breakdown and as such accelerate the rate of recovery.

With the continued provision of amino acids and exercise this culminated in a greater MPS rate in the eccentrically exercised limb 48h after damaging exercise with the consumption of a pea protein supplement. The greater anabolic response coincided with the timepoint with the greatest reduction in muscle function but the biggest progression in recovery rate from 48-72hours in the VGP condition (~9% increase in muscle function), suggesting that elevated rates of MPS over the days following eccentric exercise, with continued protein ingestion and exercise stimuli [41], could be attributed towards the repair and remodelling associated with recovery following strenuous exercise [35, 78, 186]. This delayed peak has

similarly been seen in mice models with peaks in MPS 48h following injury to the muscle [55]. The suggestion that the repeated concentric exercise bouts stimulate muscle protein turnover has been reflected in untrained individuals whereby following an initial exercise bout MPS was upregulated at 24h, with further resistance exercise bouts this cumulated to greater integrated rates of MPS over 48h (0-48h: ~0.08%/h^{-1}) [115]. Beyond this time frame, it has been demonstrated that MPS rates decline without the continued provision of amino acids [78] as well as muscle protein breakdown declining, such that fractional breakdown rate following exercise return to basal levels 48h post exercise [88]. Thus supporting that there may be a key anabolic time frame post eccentric exercise whereby the provision of exogenous amino acids is beneficial to post exercise recovery [183]. Within this chapter, we therefore show that MPS may be associated with functional recovery over the acute days (0-48h). We further extend and support existing data that it appears that the improvement in recovery of muscle function after eccentric exercise is not modulated by elevated MPS in the initial 24hours following eccentric exercise, however we demonstrate that with the continued provision of amino acids and exercise, the skeletal muscle sensitivity to amino acids remains high such that MPS rates are greater over 48hrs.

In conclusion, we utilised a pea protein nutritional supplement to determine the association between myofibrillar protein synthetic response, the recovery of muscle function, soreness and swelling following eccentric exercise. We show that pea protein supplementation, providing an additional 24.8g protein (2.2g leucine) daily, does not support functional recovery following high volume eccentric exercise and that within the first 24h whereby muscle function was lowest, MPS did not dictate functional recovery. However we demonstrate that

over 48hr of recovery, MPS was greater with the continued provision of amino acids and exercise following eccentric exercise, suggesting a key time frame in post exercise recovery whereby additional protein is beneficial.

6 Characterising the response to plant-based protein nutrition in the immediate hours post eccentric exercise

'Devon- where the jam goes first.'

The data presented in this chapter is part of a larger clinical trial. Data collection and analysis was undertaken by Kiera Wilkinson and Amy Booth. Placebo group data within this chapter has been included in a PhD thesis submitted to the University of Exeter by Amy Booth to address a separate research question.

6.1 Introduction

Chapter 4 demonstrated an accelerated recovery of muscle function over 24-72hrs following an established bout of 300 maximal unilateral eccentric contractions alongside the ingestion of 20-25g protein supplement providing ~2g leucine. This accelerated rate of recovery may have been underpinned by elevated rates of MPS induced by the additional provision of protein nutrition such that this increased muscle protein turnover at the site of injury can accelerate muscle functional recovery, however this was suggested to occur at an earlier time frame. Consequently, **chapter 0**, explored if the initial 24 to 48h post exercise underpinned the functional rate of recovery when utilising a plant-based protein supplement. The plant-based pea protein supplement, utilised in **chapter 0**, did not accelerate or support recovery over the acute days following eccentric exercise compared to a carbohydrate placebo. However, greater rates of MPS over 48hrs of recovery may have supported an accelerated rate of post eccentric exercise recovery with the continued provision of a pea protein supplement and a daily exercise stimulus. However, there is still a gap in understanding as to whether the acute hours following eccentric exercise, underpin the rapid rate of recovery within 24h, demonstrated within **chapter 4**.

Previous work has suggested that the rate of rise, peak aminoacidemia or total amino acid availability, specifically leucinemia, are key determinants of MPS [160, 203]. As demonstrated in **chapter 3**, the rate of rate or rise, peak leucinemia and total leucine availability did not predict subsequent rates of MPS, with responses ranging from 11-264% (126.7 ±8.48%) delta change from basal MPS. **Figure 6.1** splits the data presented in **chapter 3**, into animal-derived protein sources and plant-based protein sources providing 2-3g leucine. Only three

studies (4 study arms) investigating plant-based protein sources met the criterion for the systematic review, however these studies allude that inferior aminoacidemia from a plant-based protein source may not result in a reduced anabolic ability, especially in the form of a protein isolate [98, 218, 230]. Increases in MPS are observably maximal over the early (0-2h) and entire (0-6h) postprandial postexercise period, such that amongst younger individuals a resistance exercise bout sensitises the muscle to amino acids to induce a maximal anabolic response [350], regardless of the postprandial leucinemic response. However, the acute postprandial post exercise response following the ingestion of pea protein, fortified with methionine, has not been extensively investigated especially following a bout of eccentric exercise.

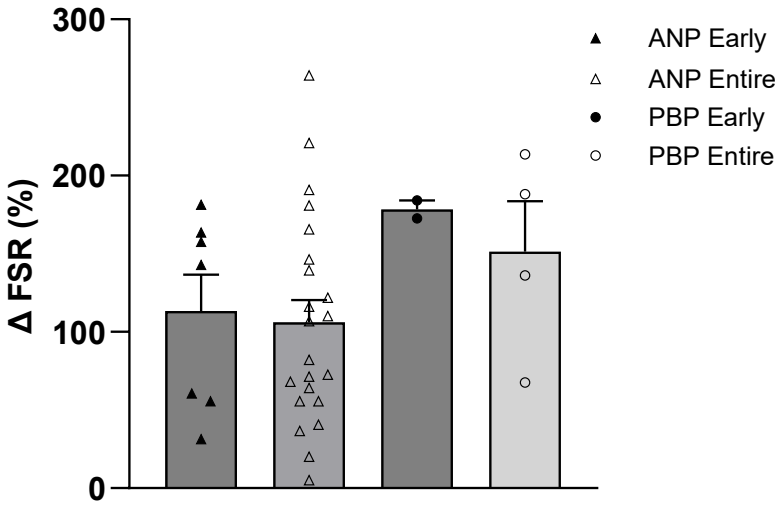

Figure 6.1 *Delta change (postprandial post-exercise increase) in muscle protein synthesis rates (MPS), early (0 - 2 h) and during the entirety of the postprandial period, expressed as fractional synthetic rate (FSR), in response to a leucine dose (2-2.6g) in all young participants (19-29 years) participants consuming either animal-based protein sources (ANP, 22 study arms) plant-based protein sources (PBP, 4 study arms).*

Myofibrillar damage increases the demand for the synthesis and remodelling of new proteins [144], the protein synthetic response in the acute hours following a high-volume eccentric exercise bout has not been extensively investigated. As demonstrated in **chapter 4** and **0**, utilising an eccentric exercise model, myofibrillar MPS exceeded responses observed utilising resistance exercise protocols however it has yet to be determined if these rates determine recovery over the acute hours post exercise. Peaks in MPS in the acute 4.5h and 6h post exercise period following an eccentric stepping bout induced a ~48% post-exercise increase in MPS without protein feeding [144]. Resisting a lever arm on an isokinetic dynamometer increased MPS by ~317% with protein feeding (45g EAA) in close temporal proximity to the exercise bout [348]. This suggests that with sufficient exogenous provision of amino acids and polyphenols, this may have a protective effect on subsequent damage inducing processes such that it provides sufficient substrate for muscle remodelling and the skeletal muscle adaptive response at the site of damage [35].

Inflammation is understood to be necessary for recovery [322], however elevated rates of inflammation can also be detrimental to the recovery process with suppression of MPS and delaying regenerative processes [25]. The process of inflammation is characterised by an early and immediate pro-inflammatory response, characterised by an increase in cytokines, such as interleukin-6 (IL-6) and monocyte chemoattractant protein (MCP-1), which promote further tissue damage [351]. This transitions to an anti-inflammatory response, indicated by an increase in IL-10 and IL1-receptor antagonist (IL1RA), which limit muscle protein breakdown and initiate structural regeneration [352]. Jameson, Pavis [186] demonstrated that an animal-derived protein polyphenol intervention suppressed

pro-inflammatory signalling cascades. Notably, this was associated with an accelerated recovery following eccentric exercise. These findings suggest a synergistic interplay between protein and polyphenols, fostering an expedited recovery process by subduing the exacerbation of inflammation and mitigating muscle protein breakdown. Consequently, this protective effect may prevent subsequent damage inducing processes at the site of injury without causing a substantial loss in muscle function.

We therefore aimed to characterise the postprandial amino acid availability, inflammatory and muscle protein synthetic response in the acute hours following a bout of maximal eccentric exercise in young recreationally active males and females. Alongside the ingestion of a pea protein supplement, to determine if the acute hours following strenuous exercise are associated with any improvement in muscle function. We hypothesised that the ingestion of a pea protein supplement will improve the functional ability of the muscle following strenuous exercise and this would be associated with a greater postprandial post exercise muscle protein synthetic response over 3 hours of recovery.

6.2 Methods

6.2.1 Participants

Participants included in this chapter are consistent with those detailed in **Chapter 5**, such that they were recruited and took part in one experimental study. The study was approved by the University of Exeter Sport and Health Sciences ethics committee (Ref No. 190703-B-01) and registered at Clinicaltrials.Gov (ID: NCT04156386).

Nineteen young, healthy recreationally active males (*n* =8) and females (*n* =11) volunteered to take part in the present study (age: 22 ±0.7 y, height: 171.1 ±2.0

cm, body mass: 64.4 ±2.14 kg, BMI 21.9 ±0.51kg·m^{-2}). Participant characteristics are displayed in **Table 6.1**.

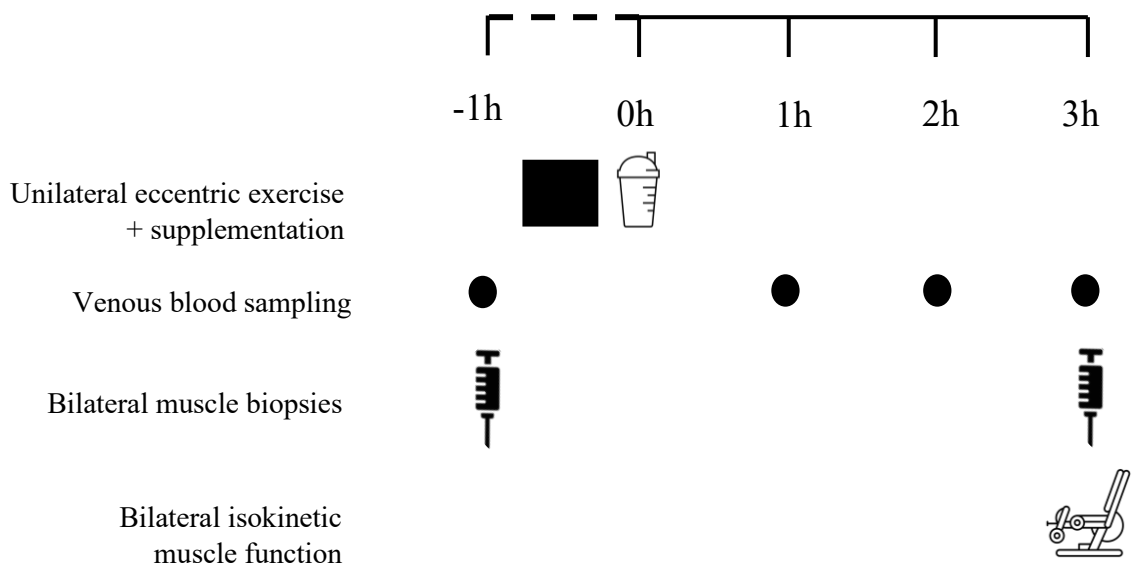

Figure 6.2 *Schematic representation of the experimental protocol. Unilateral eccentric exercise performed at ~09:00 (*t *= 0), followed by supplementation of either a vegan pea protein supplement (VGP) or maltodextrin placebo (PLC). Bilateral muscle biopsies obtained at time points 0 and 3 h post eccentric exercise in both the eccentrically exercised leg and the contralateral control leg. Blood sampling performed pre-eccentric exercise (*t *= 0) and 1,2 and 3h following exercise. Muscle function (bilateral) and soreness (visual analogue scale; VAS) test performed 3h post eccentric exercise. Full dietary control providing 1.2g·kgBM·day^{-1} dietary protein was employed for 7 days prior to the eccentric exercise bout test day.*

6.2.2 Experimental protocol

A schematic overview of the experimental protocol is shown in **Figure 6.2**. All participants completed a 7 day study period whereby they underwent strict dietary control and daily supplementation. Participants were randomly assigned to one of two double blinded parallel groups, consisting of either a daily vegan pea protein (VGP) or placebo (PLC) drinks (*see **Experimental drinks**).* Baseline measures were taken on day 1; total isokinetic work from 1 x 30 maximal

concentric contractions of the knee extensors was used to measure muscle function in both legs. Muscle function was subsequently measured 3 h following a single bout of unilateral maximal eccentric contractions (Day 7). Eccentric contractions were performed in one leg (ECC), randomly assigned and counterbalanced for leg dominance. The contralateral leg was the within participant control (CON). Immediately following the eccentric exercise bout, the supplement was consumed, and participants remained in the laboratory, resting in a semi supine position, for a subsequent 3 h. Venous blood samples were taken to determine amino acid and cytokine concentrations and bilateral muscle biopsies of the vastus lateralis were taken immediately pre (0h) and 3 h following eccentric exercise (post-exercise postprandial 0-3h recovery period). Myofibrillar protein synthesis rates in the ECC leg and CON leg were determined.

6.2.3 Familiarisation

All participants were familiarised with the exercise equipment and protocols at least 48 hours prior to commencing the experimental protocol. Muscle function test was completed and five eccentric contractions on each leg were performed. All exercise was performed on a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Shirley, New York, USA). Participants were seated with shoulder, hip and thigh straps to remove extraneous movement. The Biodex configuration was recorded during the familiarisation visit and remained the same for each subsequent visit.

Table 6.1 *Participant characteristics*

	PLC (*n=9, 6F, 3M*)	VGP (*n=10, 5F, 5M*)
Age (y)	22 ±1.1	22 ±0.9
Height (kg)	169.2 ±2.5	172.8 ±3.1
Weight (cm)	62.1 ±3.1	66.4 ±2.9
BMI ($kg.m^{-2}$)	21.7 ±0.8	22.2 ±0.6
Habitual protein intake ($g{\cdot}kgBM^{-1}{\cdot}day^{-1}$)	1.5 ±0.3	1.3 ±0.1
Controlled diet protein intake (excluding supplement) ($g{\cdot}kgBM^{-1}{\cdot}day^{-1}$)	1.2 ±0.01	1.2 ±0.01
Controlled diet protein intake (including supplement) ($g{\cdot}kgBM^{-1}{\cdot}day^{-1}$)	1.2 ±0.01	1.6 ±0.02 *
Baseline work CON leg (J)	2101 ±311	2013 ±171
Baseline ECC leg (J)	2151 ±287	2095 ±158

Values represented as mean ±SEM. PLC; daily maltodextrin placebo, VGP: daily vegan protein supplement; BMI: body mass index. Habitual protein intake recorded with a 3-day food diary. Controlled diet protein intake relative to body mass per day with and without the supplementation. ECC total work represents the total volume of eccentric work performed during the 300 eccentric contractions performed in the randomised dominant or non-dominant leg. Baseline work represents the total volume of work done in the muscle function test in the ECC; eccentrically exercised leg and the CON: control leg. * Difference between VGP and PLC in total daily protein intake

6.2.4 Eccentric exercise bout

At ~09:00am on day 7, participants performed 300 (10 sets x 30 repetitions) voluntary maximal, unilateral (ECC leg) isokinetic eccentric contractions of the knee extensors. This volume of eccentric contractions has previously been shown to impair muscle function [78, 186]. Each contraction was performed at $60°{\cdot}sec^{-1}$ over an 80° range of motion. Each set was separated by 120 seconds of rest. Participants were instructed to resist the movement maximally and were provided with verbal encouragement throughout.

6.2.5 Muscle function

Muscle function was measured on day 1 at ~08:00am (baseline) and on day 7 three hours post eccentric exercise. After arriving overnight fasted to the laboratory participants completed the muscle function test, which consisted of 30 voluntary maximal, unilateral isokinetic contractions of the knee extensors, completed on both the ECC leg and CON leg. Contractions were performed at 75°·sec^{-1} through an 80° range of motion equidistant from voluntary maximal knee extension and flexion and were preceded by a 5-repetition submaximal warm-up. Verbal encouragement was provided throughout the exercise.

6.2.6 Experimental drinks

Drinks were provided by the manufacturer (Beachbody LLC, Santa Monica, California, USA) in sachets which contained a single serving of supplement. These sachets were coded to ensure that double blinding was maintained. Vegan pea protein (VGP) and isocaloric maltodextrin (PLC) supplements were analysed for macronutrient and amino acid composition (Premier Analytical Services, High Wycombe, Buckinghamshire, UK). The pea protein supplement was fortified with methionine to raise the levels to meet the amino acid requirement per dose of protein (mg/g protein) [206]. The nutritional content of the supplements are displayed in **Table 2.1** and the contribution of the supplements to the protein content of the controlled diet is presented in **Table 2.2**. All supplements were prepared and consumed either at home in the morning (~08:00-09:00am, day 2-6) or under supervision in the laboratory immediately following exercise (day 1 and 7). Sachets were mixed with 225ml water, and the bottle was rinsed with an additional 25ml of water to ensure that all the contents were consumed. All drinks

were well tolerated, consumed within the allotted time (i.e., 5 minutes), and resulted in no reported adverse effects during or after the experimental period.

6.2.7 Dietary control

During the 7 day experimental period, participant's diet was fully controlled. All meals and snacks were provided although, water was allowed ad libitum. The diet was prepared by the research team within the nutrition kitchen of the Nutritional Physiology research unit, University of Exeter. All food was individually weighed, packaged and provided with instructions of how to prepare each meal. Energy requirements were calculated using the Henry equation [253] multiplied by an activity factor of 1.6. Daily protein intake was standardised to 1.2g.kg body mass^{-1}·day^{-1} (~15% energy contribution), with the remaining energy being contributed by fat and carbohydrates. Compliance with the controlled diet was assessed by participants returning completed daily food logs, empty food containers and daily communication.

6.2.8 Deuterated water

The deuterated water dosing protocol consisted of one loading day (day 4) and 3 maintenance days to achieve and maintain 0.6% body water deuterium enrichment. On day 4, participants arrived at the laboratory overnight fasted (~08:00am) and consumed 6ml·kg body mass^{-1} of deuterated water (70 atom %: Cambridge Isotope Laboratories Inc, Tewksbury, MA, USA) across 8 doses spread over 1.5 hour intervals. Following the first dose, participants were provided with their daily supplement and breakfast. Participants remained in the laboratory until after their fourth dose was consumed and then returned home to consume the remaining four doses. For the maintenance days (days 5-7),

participants consumed a daily dose of 0.54mL·kg body mass^{-1} 70% deuterated water upon waking.

6.2.9 Muscle biopsy collection

Muscle biopsies were collected from the mid region of the *m. vastus lateralis* (approx. 15cm above the patella) with a modified Bergstrom needle under local anaesthesia (2% lidocaine). All biopsy samples were immediately freed from any visible blood, adipose or connective tissue, and were frozen immediately in liquid nitrogen and stored at -80°C until subsequent analysis. Muscle tissue collection occurred before (-1 h) and 3 h after the bout of maximal unilateral eccentric exercise.

6.2.10 Blood sample collection

A venous blood sample, from the antecubital vein was collected on day 1 and 7 from participants when they arrived overnight fasted (~08:00am). Following exercise, an 18-gauge cannula was inserted in the antecubital vein for repeated blood sampling; 1, 2 and 3 h post-exercise postprandial.

6.2.11 Blood sample analysis

Blood glucose concentrations

One mL of whole blood was immediately analysed for glucose concentrations (Biosen C -line, EKF diagnostics, Cardiff, UK)

Serum insulin concentrations

Serum insulin concentrations were determined using a commercially available ELISA assay kit (DRG Insulin ELISA, EIA-2935, DRG Instruments Inc.).

Body water deuterium enrichment

Body water deuterium enrichment was measured using plasma samples collected at baseline and day 7 of the study, at the University of Texas Medical Branch. A ThermoFisher Delta V Advantage Isotope Ratio mass spectrometer (IRMS) (Bremen, Germany), equipped with a Finnigan GasBench II (Thermo Fisher Scientific, Waltham, MA, USA), was used for stable hydrogen isotope ratio measurements. See ***methods section 2.4.4***

Plasma amino acid concentrations

Plasma amino acid concentrations (leucine, valine, isoleucine, phenylalanine, lysine, histidine, glycine, glutamic acid, methionine, proline, serine, threonine, tyrosine and alanine) from baseline, 1, 2 and 3 hours following eccentric exercise and consumption of experimental drink were determined by GC-MS in tert-butyldimethylsilyl derivatives [136]. See ***methods section 2.5.4***.

Plasma cytokine concentrations

The plasma concentrations of interleukin (IL)-6, -10, -1RA, monocyte chemoattractant protein-1 (MCP), macrophage colony stimulating factor (MCSF), tumor-necrosis factor related apoptosis-inducing ligand (TRAIL) were measured in blood plasma in duplicate using a multiplex assay according to manufacturer instructions using the manufacturer supplied reagents, antibodies and the MESO QuickPlex SQ 120 instrument (U-PLEX, Meso Scale Diagnostics LLC (MSD), Rockville, MD, USA). See ***methods section 2.5.5.***

6.2.12 Muscle tissue analysis

Myofibrillar Protein-bound [^{2}H] alanine enrichment

The myofibrillar fraction of skeletal muscle tissue was extracted to be able to determine the enrichment of myofibrillar protein-bound [^{2}H] alanine. See ***methods section 2.5.6***.

6.2.13 Calculations

Myofibrillar protein fractional synthesis rates were calculated based on the incorporation of [^{2}H]alanine into myofibrillar protein and mean plasma deuterium enrichment between muscle biopsy timepoints using the following standard precursor-product equation:

$$FSR\ (\%{\cdot}h^{-1}) = \frac{\Delta E\mathrm{p}}{3.7\ \mathrm{x}\ Eprecursor\ \mathrm{x}\ t}\ X\ 100$$

where Δ*E*p is the increment in [^{2}H]alanine enrichment in myofibrillar protein between two (0 and 3 h) biopsies, $E_{\mathrm{precursor}}$ is the average body water deuterium enrichment between two biopsies corrected by a factor of 3.7 based upon the deuterium labelling of alanine during *de novo* synthesis, and *t* indicates the tracer incorporation time between two muscle biopsies.

6.2.14 Statistical analysis

Differences in subject characteristics, dietary intake and total eccentric isokinetic work between conditions were analysed using unpaired t-tests. A repeated measure 2-factor analysis of variance (ANOVA) was used to analyse (treatment x time) differences in plasma amino acid and cytokine concentrations, body water ^{2}H enrichment, delta change isokinetic concentric work and myofibrillar fractional synthetic rate (FSR). Repeated measure 3-factor ANOVA was used to analyse (time x leg x condition) differences in myofibrillar bound [^{2}H] alanine enrichment. When a significant main and interaction effect was observed, a Sidak post hoc test was performed to locate individual differences. Calculations were performed

using GraphPad Prism 9.5.1. Statistical significance was set at $P < 0.05$. All data are expressed as means ±standard error of the mean (SEM).

6.3 Results

6.3.1 Participant characteristics

No differences in age, body mass, height, BMI or habitual nutritional intake were detected between groups (**Table 6.1**, $P > 0.05$). No differences in total work performed during the unilateral bout of eccentric exercise were detected (**Figure 6.7A**, $P > 0.05$).

6.3.2 Plasma amino acid, glucose and serum insulin concentrations

Plasma total amino acids (TAA), essential amino acids (EAA), branched chain amino acid (BCAA), non- essential amino acids (NEAA), leucine and methionine concentrations post eccentric exercise and following the consumption of the supplementation beverage are shown in **Figure 6.3** and **Figure 6.4**. No differences between groups were seen at baseline (pre -exercise and postabsorptive, $P > 0.05$), however the influence of feeding and eccentric exercise led to differences between groups across all other time points for all plasma amino acid concentrations presented, except NEAA (condition x time interaction, all $P < 0.001$). Plasma TAA (**Figure 6.3A**) increased 1 h post -exercise postprandial, after the consumption of VGP supplement, peaked at 2354.47 ±153.97μmol/L, and decreased back to baseline 2-3h post-exercise ($P > 0.05$). Following PLC supplementation, plasma TAA declined over 1 and 3 hours post-exercise postprandial, with no differences to baseline 2h post exercise, however remained lower than baseline at 3 h postprandial.

Plasma NEAA (**Figure 6.3B**) following eccentric exercise and VGP consumption was no different to baseline over the 3 h time frame, however in the PLC condition

NEAA declined over 1 and 3 h compared to baseline concentrations. Plasma EAA (**Figure 6.3C**) and BCAA (**Figure 6.3D**) significantly increased from baseline 1h post exercise postprandial following VGP supplementation and returned to baseline values by 2 h and by 3h, respectively. However post eccentric exercise and the consumption of the PLC supplementation a decline in plasma EAA and BCAA concentrations was seen 1, 2 and 3h post -exercise postprandial (*P* <0.01).

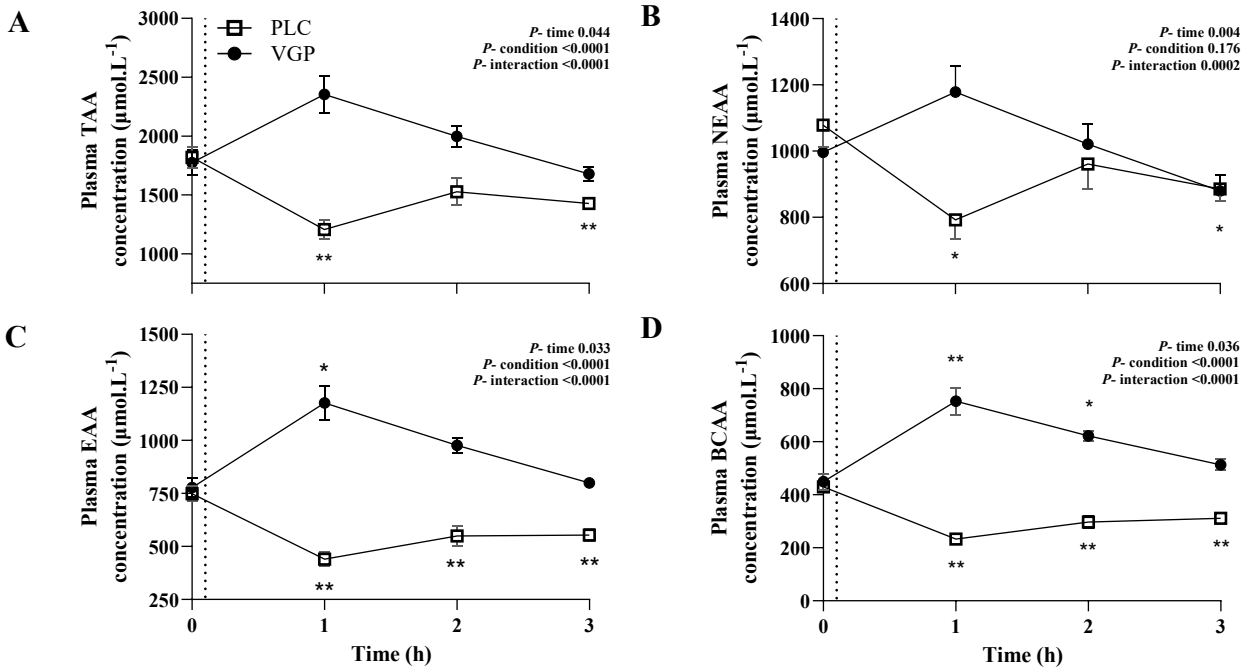

Figure 6.3 *The time course of plasma total amino acids (TAA, **A**), non-essential amino acids (NEAA, **B**), essential amino acids (EAA, **C**), branched chain amino acids (BCAA, **D**) concentrations during the a 3 h post-exercise postprandial period in healthy recreationally active males and females, following the consumption of a vegan pea protein supplement (VGP, n = 10) or a maltodextrin placebo (PLC, n = 9) consumed immediately after 300 unilateral maximal eccentric contractions (represented by dashed line). Statistical analysis was performed with a two-factor ANOVA. Data are presented as means and error bars represent standard error (SEM). ** Means different from baseline (P <0.01). * Means different from baseline (P <0.05)*

Plasma leucine concentration (**Figure 6.4A**) significantly rose in VGP (1h; 264.10 ±18.79 μmmol/L) and remained higher than baseline until 2h post-exercise postprandial. This response was reflected in the PLC group with a significant decline in plasma leucine from baseline across all postprandial time points (baseline: 129.38 ±7.06, 1 h; 64.04 ±4.63 μmmol/L, $P < 0.001$).

Plasma methionine concentration (**Figure 6.4B**) significantly rose following eccentric exercise and VGP supplementation and was significantly elevated after 1h, with a peak of 35.64 ±2.91 μmmol/L post-exercise postprandial. This response was reflected in the PLC group with a significant decline (baseline; 20.33 ±1.17, 1h; 11.53 ±1.07 μmmol/L) in plasma methionine from baseline across all postprandial time points ($P < 0.001$), resulting in a significant difference between PLC and VGP treatment groups ($P <0.0001$).

Plasma glucose concentrations during the post-exercise postprandial period are displayed in **Figure 6.5A.** From similar fasting concentrations (PLC: 4.01 ±0.16μmol/L and VGP: 3.95 ±0.48μmol/L, $P = 0.066$), there was an increase in plasma glucose concentration in the VGP condition and a decrease in plasma glucose concentration in the PLC condition over 1 h, however despite these differences plasma glucose did not differ between groups across any time point (main condition effect, $P > 0.05$). However there was a significant *time x condition* interaction and both PLC and VGP plasma glucose concentrations significantly changed between 1 and 2 h postprandial.

Serum insulin concentrations are displayed in **Figure 6.5B**. Fasting insulin concentrations were similar between treatment groups (PLC; 4.01 ±0.16μmol/L and VGP; 3.95 ±0.14μmol/L, $P = 0.941$). Similar responses were seen for both VGP and PLC (main condition effect, $P = 0.644$) with increasing serum insulin

concentrations over 1 h postprandial post eccentric exercise (main time effect, *P* <0.0001). After peaking 1h postprandial, insulin concentrations declined, however remained different to baseline after 3 h (*P* = 0.033).

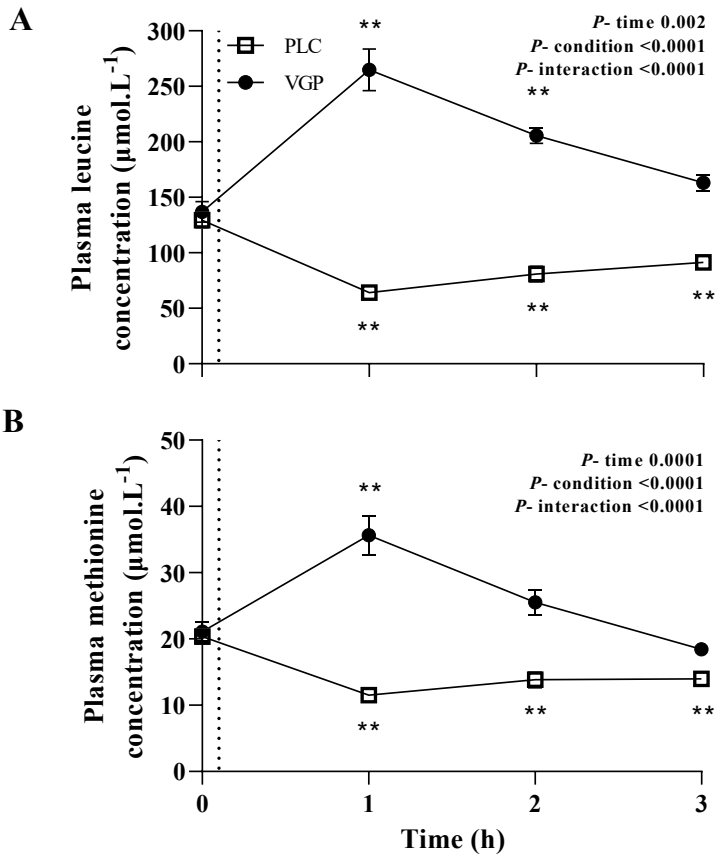

Figure 6.4 *The time course of plasma leucine (**A**) and methionine (**B**) concentrations during the 3 h post-exercise postprandial period in healthy recreationally active males and females, following the consumption of a vegan pea protein supplement (VGP, n = 10) or a maltodextrin isocaloric placebo (PLC, n = 9) consumed immediately after 300 unilateral maximal eccentric contractions (represented by dashed line). Statistical analysis was performed with a two-factor ANOVA. Data are presented as means and error bars represent standard error (SEM). ** Means different from baseline (P <0.01).*

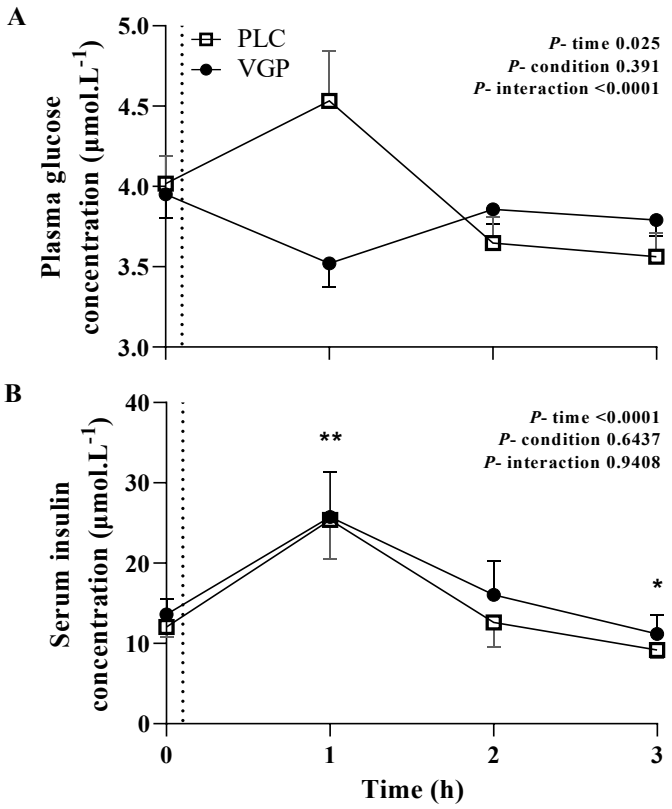

Figure 6.5 *Plasma glucose and serum insulin concentrations during the 3 h post-exercise postprandial period in healthy recreationally active males and females, following the consumption of a vegan pea protein supplement (VGP, n = 10) or a maltodextrin isocaloric placebo (PLC, n = 9) consumed immediately after 300 unilateral maximal eccentric contractions (represented by dashed line). Statistical analysis was performed with a two-factor ANOVA. Data are presented as means and error bars represent standard error (SEM). ** Means different from baseline (P <0.01). * Means different from baseline (P <0.05)*

6.3.3 Plasma cytokine concentrations

Plasma cytokines (**Figure 6.6Error! Reference source not found.**) were measured 1h and 3h post exercise postprandial. There were no differences between PLC and VGP conditions on plasma cytokine concentrations (main condition effect *P* >0.05) following eccentric exercise and supplementation. IL-6,

MCSF and TRAIL all changed over the acute period post-exercise postprandial compared to baseline (pre dietary intervention, main time effect, *P* <0.0001). Plasma IL-6 concentration increased compared to baseline 3 h post- exercise for both the PLC and VGP condition. Plasma MCSF and TRAIL concentrations decreased following eccentric exercise compared to baseline over 1 and 3h post exercise for both PLC and VGP condition.

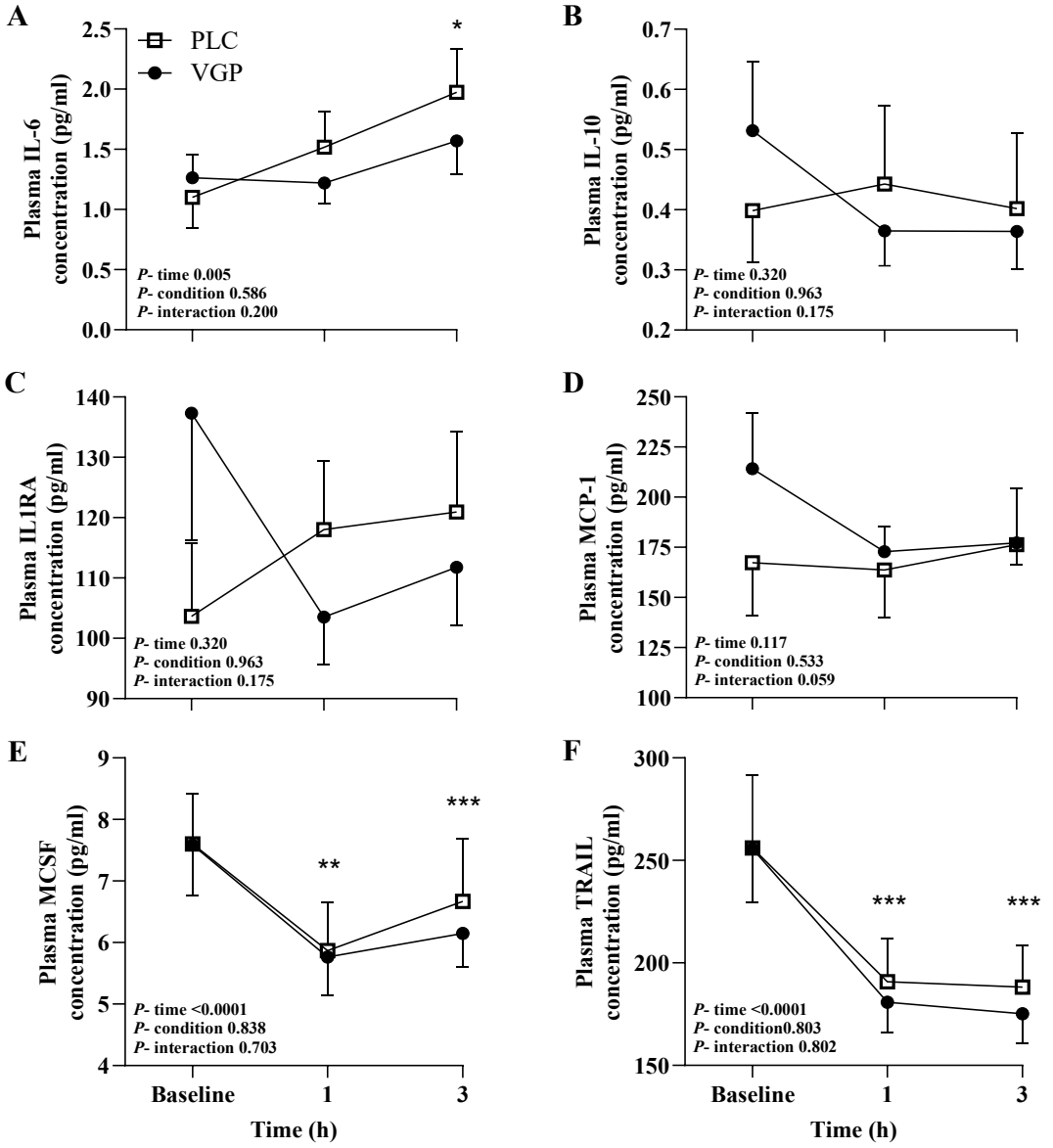

Figure 6.6 *Plasma cytokine concentrations measured baseline (pre dietary control) and 1h and 3h following 300 maximal unilateral eccentric contractions immediately followed by the consumption of a vegan pea protein supplement*

*(VGP, n = 10) or a maltodextrin isocaloric placebo (PLC, n = 9). Data are presented as means and error bars represent standard error (SEM). Statistical analysis was performed with a two-factor ANOVA. * Means different from baseline (P < 0.05). ** Means different from baseline (P < 0.05).*

6.3.4 Muscle function

The difference (delta change) between baseline and 3 h post eccentric exercise muscle function, measured as total work over 30 maximal isokinetic concentric contractions in both the eccentrically exercise leg (ECC) and the contralateral control (CON) is shown in **Figure 6.7B**. Delta change total isokinetic work was significantly different between legs (main leg effect, *P* <0.01) with the greatest decline in muscle function in the ECC leg (PLC ECC: -463 ±119.24 J and VGP ECC: -246 ±60.69 J). The PLC CON leg displayed a decline in muscle function 3 h following eccentric contractions which were performed in the contralateral leg. The VGP CON leg showed an increase from baseline (2012.83 ±171.22 J) in total isokinetic work done 3 h post eccentric exercise performed in the contralateral leg (2132.22 ±184.64 J). There was a significant difference between PLC and VGP delta change muscle function (*P* = 0.024), however post hoc analysis revealed no differences between treatment groups.

6.3.5 Plasma deuterium, myofibrillar protein enrichment and fractional synthesis rates

Plasma deuterium enrichment was -0.01 ±0.002% and -0.02 ±0.004% at baseline in PLC and VGP respectively. At time point 0 h (pre-exercise) plasma deuterium enrichment was 0.55 ±0.04% and 0.56 ±0.04% in PLC and VGP, respectively (*P* >0.05). Post exercise (3 h) plasma deuterium enrichment reached 0.60 ±0.03% and 0.60 ±0.04% at 3h post exercise in PLC and VGP (*P* >0.05).

Myofibrillar Protein-bound [^{2}H] alanine enrichment increased over the post-exercise postprandial period, shown in **Figure 6.8A** (0-3 h, time effect *P* <0.0001). Protein bound alanine increased in the PLC condition by 25.82 ±6.80% in the CON leg and 31.08 ±11.60% in the ECC leg, over 3 h. In the VGP condition protein-bound [^{2}H] alanine enrichment increased by 25.50 ±5.93% in the CON leg and 27.02 ±9.85% in the ECC leg. However there were no differences found between legs (CON vs. ECC; main leg effect P > 0.05).

Post -exercise postprandial myoFSR is displayed in **Figure 6.8B**. Acute post exercise (0-3h) myoFSR was similar between the CON versus ECC in both PLC and VGP group (PLC: 0.123 ±0.046 vs. 0.151 ±0.071; VGP: 0.241 ±0.090 vs. 0.288 ±0.098%·h^{-1}; leg x treatment interaction *P* > 0.05). There was a trend for protein ingestion (VGP group) to induce a greater postexercise postprandial myoFSR (*P* = 0.084), irrespective of exercised leg.

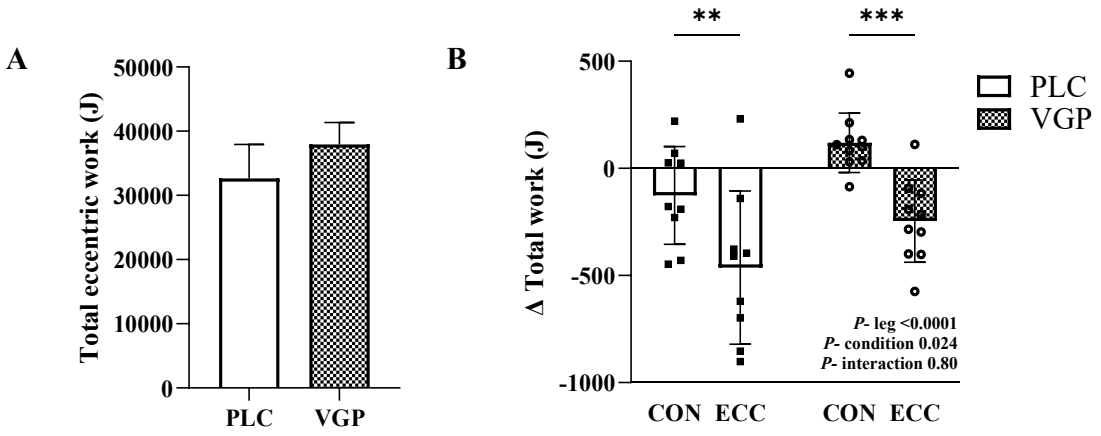

Figure 6.7 *Total eccentric isokinetic work completed during 300 maximal unilateral eccentric contractions* ***(A)*** *and Total isokinetic work* ***(B)*** *completed during a muscle function test consisting of 1 x 30 maximal concentric contractions of the knee extensors expressed as delta change between baseline muscle function test and 3 h following 300 maximal unilateral eccentric contractions in*

*the ECC leg and the CON leg rested. Data are presented as means and error bars represent standard error (SEM). Statistical analysis was performed with an unpaired t-test (**A**) and a two-factor ANOVA (**B,** leg x treatment). *** Means different between legs (P < 0.001), ** means different between legs (P < 0.01).*

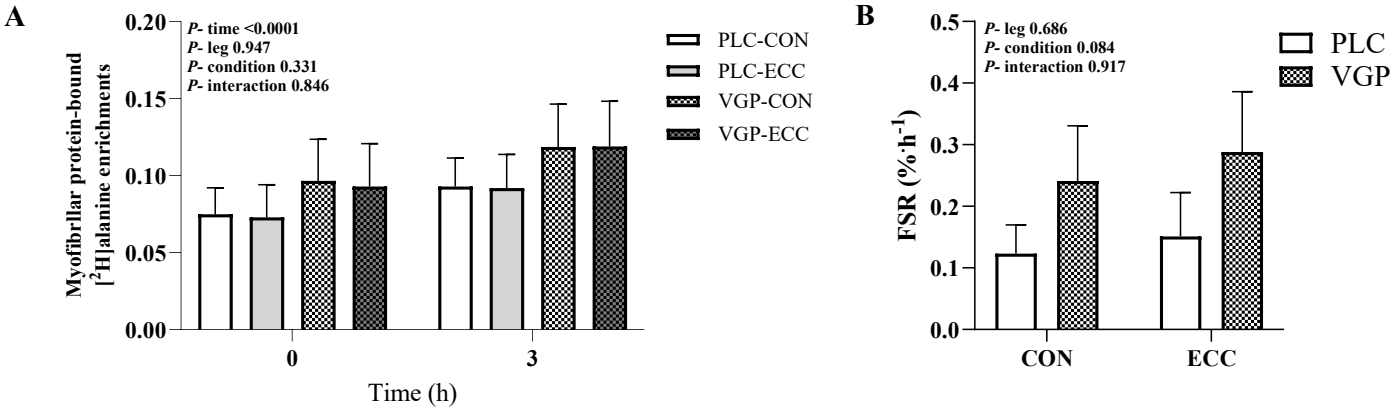

Figure 6.8 *Myofibrillar bound [^{2}H] alanine enrichment (**A**) and acute (0-3 h) myofibrillar fractional synthetic rate (FSR) (**B**). At time 0, 300 maximal unilateral eccentric contractions were performed and a vegan pea protein supplement (VGP, n = 10) or a maltodextrin isocaloric placebo (PLC, n = 9) were immediately consumed following exercise. Data are presented as means and error bars represent standard error (SEM). Statistical analysis was performed with a three-factor ANOVA (**A**, time x leg x condition) and a two-factor ANOVA (**B**, leg x condition).*

6.4 Discussion

The primary objective of **Chapter 6** was to determine if the postexercise muscle protein synthetic response dictates any functional changes in the acute hours following high volume maximal eccentric exercise in a cohort of recreationally active males and females. We used an established unilateral eccentric exercise model to initiate an inflammatory response and induce an impairment in muscle function, to investigate whether a plant-based protein supplement can attenuate the extent of muscle damage and modulate functional recovery from strenuous eccentric exercise. We hypothesised that the ingestion of a pea protein

supplement, providing 24.8g protein and 2.2g leucine, would improve the functional ability of the muscle following eccentric exercise and this would be associated with a greater postprandial post exercise muscle protein synthetic response over 3 hours of recovery. Amongst recreationally active participants, following 300 unilateral eccentric contractions, the ability to produce force was compromised 3h following exercise with a reduction in muscle function of 24% and 18% seen in PLC and VGP conditions, respectively (**Figure 6.7B**). We observed an acute cytokine mediated inflammatory response to eccentric exercise with increases in IL-6 concentrations over 3 h across both conditions (Error! Reference source not found.**A**), suggestive of primary muscle damage w hich may contribute to the loss in force generating capacity [25]. Interestingly, VGP supplementation allowed more work to be done 3h following eccentric exercise in the contralateral control leg (CON) and the reduction in muscle function was less severe in the ECC leg. The ability to produce more isokinetic work may be underpinned by the trend for VGP to elicit a heightened MPS response in contrast to the PLC condition.

We had suggested that with the addition of sufficient exogenous provision of amino acids and polyphenols this may have a protective effect on subsequent damage inducing processes or that it provides sufficient substrate for muscle remodelling and the skeletal muscle adaptive response [35]. Such that at the site of muscle damage, muscle protein turnover is increased, therefore sufficient substrate is required to allow this effective remodelling. The blood sampling time points may not have captured the peak or total exposure of amino acid concentrations but we can be confident that it captures the postprandial aminoacidemia, indicative of a rise in amino acids following the consumption of 24.8g pea protein isolate, with comparable peaks in total aminoacidemia of

~2500μmol/L seen 60mins following the consumption of 25g pea protein [245] and leucinemia (~250μmol/L) following the consumption of wheat and milk protein [229]. We aimed to mitigate the deficiency in pea protein, by fortifying with methionine, raising to 2% (0.5g) of the total protein, which raises the contribution above what is recommended for a whole protein source (1.6%) [206]. The provision of 0.5g methionine, led to a 59% rise in methionine availability over 60mins (**Figure 6.4B**). In contrast, when a 25g pea protein isolate providing 0.2g (1.25%) methionine was consumed, a rise of 13% 60min following whole body resistance exercise, resulted in a negative postprandial total exposure to methionine, which is suggestive of a heightened demand for methionine [245]. Such that by fortifying with methionine we adequately raised the availability of an essential amino acid to improve the overall quality of the post exercise supplement. Despite **Chapter 3** demonstrating that leucine *per se* is not the prime determinant of post-exercise anabolic responses and discussing that plant based protein sources with lower EAA content and incomplete AA profiles can effectively increase MPS in healthy young adults [98, 218, 229, 245], the aminoacidemia seen following the consumption of the pea protein consumption would provide sufficient substrate for remodelling.

The substrate availability following pea protein consumption, resulted in a trend for elevated muscle protein synthesis in comparison to a carbohydrate placebo. Acute postprandial post resistance exercise rates of MPS are commonly ~0.06-0.08%/h^{-1} [170], whereas here we present rates of 0.1-0.3%·h^{-1} after high volume maximal eccentric exercise and both the consumption of 24.8g protein and 31.8g maltodextrin placebo. Over 4.5h and 6h following high volume eccentric exercise MPS responses, without the addition of exogenous protein, were ~0.110%·h^{-1}[144] and ~0.126%·h^{-1} [348], respectively. At face value, these results

demonstrate that a pea protein isolate is an effective nutritional intervention to enhance rates of MPS following eccentric exercise such that this provides a protective effect on subsequent damage inducing processes or that it provides sufficient substrate for muscle remodelling and the skeletal muscle adaptive response [35], so that the extent of the initial muscle damage is less severe as demonstrated by a greater ability to produce isokinetic work following VGP supplementation.

The myofibrillar fractional synthetic rates we present across both conditions within this chapter are greater than previously shown over the acute hours following eccentric exercise (PLC, 0.241 ±0.090, VGP: 0.288 ±0.098%·h^{-1}) which indicates a high demand for amino acids following strenuous muscle contractions. This high demand is demonstrated by postprandial aminoacidemia decreasing following eccentric exercise in the PLC condition (**Figure 6.3**), suggesting that the amino acid demand is high and clearance from the blood plasma is high in the acute hours following the bout of strenuous exercise, insinuating a reliance on endogenous amino acids to be supplied for repair and remodelling [35]. Thereby, the high demand for amino acids may be met with elevated rates of muscle protein breakdown (MPB). The inward transport of amino acids from MPB into the muscle cell can contribute to muscle anabolism by increasing the intracellular amino acid availability for MPS [137, 349, 353]. The simultaneous rise in MPS and MPB results in increased total protein turnover at the injury site. The provision of additional amino acids from a protein supplement (VGP), could positively impact the immediate post-exercise period by mitigating skeletal muscle protein breakdown. This is indicated by a trend for greater MPS rates in the VGP supplement MPS response, suggesting a protective mechanism against

an initial significant decline in muscle function. However, the extent of muscle protein breakdown may limit a clear positive influence from the VGP supplementation, as it provides substrate for elevated MPS rates in both ECC and CON limbs within the PLC condition also. It remains unclear if the direct provision of exogenous amino acids determined the MPS response, leading to increased turnover at the injury site and facilitating enhanced muscle function 3 hours after eccentric exercise. However, as indicated in **Chapter 5**, the prolonged supply of amino acids during the post-exercise recovery period enables sustained and heightened MPS rates, possibly after MPB has returned to basal levels.

Here we demonstrate that pea protein, has a potential protective effect on reducing the extent of muscle damage in the early hours post strenuous exercise, with the ability to produce more total work (**Figure 6.7B**). Previous work has highlighted that following eccentric exercise muscle mRNA expression of tumor necrosis factor related apoptosis inducing ligand (TRAIL) receptor TNF receptor superfamily member 10B was supressed [186] and plasma concentrations of TRAIL decreased 3 to 6 h post exercise [252], which is consistent with the findings in the present study of a decreased concentration of TRAIL over 1 and 3 h post eccentric exercise (Error! Reference source not found.**F**). The d ownregulation of the upstream pathways of NF-κB has been associated with expedited recovery following eccentric exercise [186], suggestive that these pro -inflammatory pathways are involved with delaying the recovery of muscle function. The higher availability of exogenous amino acids, a suppression in circulating TRAIL concentrations, indicative of potential link to a reduction in specific inflammatory and degradation pathways and overall large rates of MPS, may stabilise the integrity of the muscle to provide a protective effect post exercise and be a mediator of muscle recovery in the acute hours, which allows

the muscle to produce more force. These mechanisms may be at play within Pavis, Jameson [78] such that function is recovered 24h following eccentric exercise with an animal -derived protein supplement and this is reflected in **chapter 0**, where we present a smaller loss in muscle function as compared to previous studies utilising the same exercise model in the placebo group [78], which we had suggested to be due to the additional exercise stimulus increasing MPS at the site of injury and supports our findings of a greater muscle protein turnover within the acute hours post exercise.

However, despite the ability to produce more isokinetic work in the acute hours following strenuous exercise, **chapter 5** presented that a pea protein supplement does not accelerate functional recovery such that function does not recover till 96h, which may be suggestive of sustained pro-inflammatory events across both conditions. We have presented an increase in IL-6 concentrations 3 h post exercise (Error! Reference source not found.**A**) indicating increases in tissue d egradation which has been associated with contributing to the loss in force generating capacity following eccentric exercise [354, 355]. Over 3h of recovery circulating concentrations of macrophage colony stimulating factor (MCSF) decreased. The presence of MCSF promotes the phenotypic switch towards M2 macrophages which induces anti-inflammatory events, myofiber regrowth and muscle force recovery, shown following limb unloading in mice [356]. With a further function of MCSF being suppressing the expression of UPS pathways [356], such that proteolysis is decreased. With the decrease in concentrations of MCSF following eccentric exercise, this is suggestive of a potential link with an increased pro -inflammatory and muscle protein breakdown state in both conditions in the acute hours following exercise, supporting the notion of an increased rate of muscle protein turnover at the sight of injury. We therefore

strongly encourage future research to quantify muscle protein breakdown over the acute recovery period, especially in the initial 24h where no differences between conditions have been shown, to determine if muscle protein turnover following eccentric exercise underpins recovery.

In conclusion, nutritional supplementation with a pea protein supplement offers substantial exogenous amino acid provision, concurrently suppressing inflammatory markers linked to NF-kB pathways that could otherwise impede recovery. This supplementation strategy induces elevated rates of MPS, collectively contributing to the stabilisation of muscle integrity during the acute hours of post-eccentric exercise recovery. This stabilisation facilitated an enhanced capacity for the muscle to perform more isokinetic work.

7 General Discussion

‘A work of art does not answer questions, it provokes them.’

Leonard Bernstein

Understanding the biochemical and physiological mechanisms of muscle damage, soreness and recovery can assist developing strategies to enhance recovery, build and maintain muscle mass, understand myopathic disease progression and the unpleasant consequences of exercise that are experienced under unaccustomed conditions. This thesis has focused on the time course of recovery and muscle protein synthesis post eccentric exercise. The primary aim of this thesis was to determine whether a novel plant-based protein supplement could accelerate recovery after eccentric exercise. Alongside determining if myofibrillar protein synthesis rates underpin functional exercise recovery, such that by promoting muscle protein synthesis (MPS), this would accelerate recovery from muscle damaging eccentric exercise.

7.1 Recovery of skeletal muscle function

If exercise is unaccustomed and strenuous, the muscle may experience damage, which may take 2-7days for the muscle to recover [25]. Various experimental models have been utilised to safely induce muscle damage, within this thesis we have utilised a voluntary unilateral maximal eccentric exercise model of the knee extensors across a large range of motion to induce muscle damage, which has been adopted by several studies [33, 34, 78, 186, 322, 331]. The assessment of muscle damage requires reliable and direct markers. The pilot methodology discussed in **Section 2.7.2**, was an attempt to observe histological alterations, using electron microscopy, to quantify myofibrillar disruption to the muscle ultrastructure. The ultrastructure changes first observed by Friden, Sjöström [28], have been linked to a reduction in force generating capacity. However whether this histological analysis is representative of the whole muscle needs to be considered. Changes in force generating capacity (muscle function) appear to be

the most valid and predominantly used marker of muscle damage [25, 339]. Other markers of muscle damage, including muscle soreness and creatine kinase (CK) levels are useful additional markers to demonstrate alterations to the state of the muscle [65].

Throughout this thesis, muscle function served as a metric to assess impairment and recovery following eccentric exercise, measured by total work during 30 maximal unilateral isokinetic contractions. **Figure 2.3** illustrates that peak torque in the last 5-10 repetitions was less than half of the initial contractions, reflecting changes in both strength and fatiguability. This impairment aligns with mechanical disruption to the muscle contractile apparatus of the muscle [31], leakage of intracellular proteins and enzymes [45], infiltration of pro inflammatory cytokines [354], activation of calpain the calcium dependent protease [31] and diminished ability for excitation-contraction coupling, as seen in reduced action potential responsiveness and force production [48]. Consistent with similar eccentric exercise protocols in other studies, an average 29 ±2% reduction in isometric torque was induced across **Chapters 4-6**, categorised as 'moderate exercise-induced muscle damage' [25]. Muscle function typically recovers completely within 7 days, whereas in this thesis, recovery occurred within 5 days post-eccentric exercise. While histological evidence of skeletal muscle damage is lacking, the ~29% reduction in muscle function and 40% increase in muscle soreness suggest increased myofibrillar disruption. Similar studies reporting a 25-50% reduction in muscle function also observed elevated serum creatine kinase activity [25], reduced immunohistochemical staining for desmin and dystrophin indicating contractile structure disruption [33], myofibrillar disruption to the

sarcomeres and contractile apparatus [31], and upregulated inflammatory pathways [78, 186].

Variability in responses among participants using the same eccentric exercise model has been observed in the literature. Raastad, Owe [31] reported a ~47% reduction in muscle function, ranging from 19-73%, attributed to uncontrolled participant activity/training status. Pavis, Jameson [78] demonstrated a 35-40% reduction in muscle function after eccentric exercise in recreationally active individuals. In **Chapters 4-6** of this thesis, the reduction in muscle function varied (10-65%), suggesting that a relatively small reduction (<20%) may indicate a protective response or possibly participants not working maximally. The initial eccentric exercise bout produced the greatest eccentric torque however this rapidly diminishes over the 10 sets of 30 repetitions, as presented in **Figure 2.2**, suggesting that the initial bouts of eccentric contractions may be where the initial damage events occur to the muscle such that, further sets are fatigue inducing rather than damage inducing in which the muscle needs to recover from. With moderate muscle damage also being experienced following 100 eccentric contractions in sedentary and recreationally active individuals [61, 193]. This suggests that amongst recreationally active participants performing 300 eccentric contractions, despite familiarisation and encouragement, may not be accustomed to maximal effort exercise, especially when fatigue sets in. In **Chapter 5**, the smaller reductions in muscle function were attributed to an additional concentric exercise bout 3 h post-initial exercise, leading to greater turnover at the injury site and smaller reduction in muscle function, as demonstrated in **Chapter 6**.

An additional indirect marker employed throughout this thesis was muscle soreness, a subjective measure relying on individuals rating their soreness levels

post-exercise. This marker serves as an indicator of damage progression associated with eccentric exercise, typically peaking over 24-48 h post exercise. In **Chapter 4**, muscle soreness peaked earlier in females than males, aligning with the greatest reductions in muscle function. The overall increase in soreness was approximately 40%, consistent with findings in **Chapter 5** and Pavis, Jameson [78]. The time course and precise mechanisms underlying delayed onset of muscle soreness (DOMS) remain uncertain [21]. Peaks in CK, myoglobin release, or morphological disruption do not align with the time course of muscle soreness. Swelling, as measured by thigh circumference [66] and intracellular pressure [71], has similarly not aligned with DOMS development. Although intramuscular pressure increased 48 hours post-exercise, the relation to muscle soreness progression was not explored [71]. MRI scans, frequently used for measuring limb and muscle volumes, particularly during immobilisation [83], have rarely been employed to assess changes following eccentric exercise. The time course of DOMS and the water content of tissue fluid utilising T1 and T2 relaxation time from MRI scans have been shown to relate to the time course of DOMS, with peaks at 24 and 48 h post exercise, however feelings of soreness where also evident before these time points suggestive of other factors acting on or aggravating pain afferents earlier [357]. In **Chapter 5**, MRI scans were used to investigate whether delayed onset of muscle soreness was linked to increased muscle swelling, indicating oedema. However, muscle volume showed no increase over 24 and 48 h post-eccentric exercise, the period when muscle soreness peaked. This may be attributed to the extent of muscle damage not causing a loss in cell membrane integrity, resulting in less severe influx and metabolic disruption. Consequently, fluid may not have entered the interstitium.

7.2 Nutritional intervention to assist with functional recovery

The area of research investigating post exercise recovery utilising protein supplementation has become an increasingly popular area of research, with a large proportion of research looking into the synergistic relationship between protein ingestion and resistance exercise. Resistance exercise which constitutes both the concentric and eccentric movement phases whilst moving an external load, has the ability to stimulate MPS rates over the acute hours (1-6 h) and this response can remain elevated for 24-48 h [150, 183], such that protein ingestion augments the rise in MPS rates [93]. The studies utilising a resistance exercise protocol and a protein supplementation in close temporal proximity to exercise were systematically reviewed in **Chapter 3,** to determine the relationship between leucine and postprandial post exercise MPS responses. It has been generally assumed that animal based protein supplements are more efficacious compared to the plant based protein counterparts at rest and postexercise [160], due to a superior amino acid profile and rapid digestion and absorption kinetics [174, 203] and as such have the ability to reach the leucine threshold and leucine trigger and stimulate maximal MPS responses. However the findings presented in **chapter 3,** challenge this notion, indicating that the leucine dose and the subsequent total peripheral availability of leucine does not predict the MPS response in young individuals following resistance exercise. Findings from Tischler, Desautels [358] determined that the leucyl-tRNA or leucine concentration does not limit translation initiation or protein synthesis, such that when there is a saturated concentration of intracellular leucine, this does not determine the extent of the anabolic stimulus. This aligns with conclusions drawn within **Chapter 3**, that we cannot ascertain a specific leucine trigger or threshold from leucine dose or plasma leucine variables, as this may not be the limiting

factor to a maximal anabolic response. This suggests that an increase in intracellular leucine might act as an initial stimulus, but it may not enhance MPS beyond what a resistance exercise stimulus alone can achieve. This aligns with an increasing disconnect between aminoacidemia, specifically leucinemia, under conditions of coingestion, whole foods or plant-based protein sources not indicating suboptimal MPS rates. **Figure 6.1** demonstrates the diverse MPS responses following exercise and protein ingestion across various studies providing 2-3g leucine, the commonly recommended dose in sports nutrition [165, 305-309]. Notably, the three studies employing plant-based protein supplement after resistance exercise within **chapter 3**, demonstrate MPS responses similar to, if not greater than, the animal-based protein sources providing 2-3g leucine. The mechanistic insight into muscle protein synthetic responses following eccentric exercise and how this influences post eccentric exercise recovery is sparse. A maximised anabolic response following damaging exercise is thought to be the mechanism that alleviates muscle soreness and accelerates recovery of muscle function following strenuous damaging exercise [59, 61, 77, 78, 127, 186, 188]. We therefore seeked to determine whether providing 20g protein and ~2g leucine would enhance the myofibrillar protein synthetic response and assist with adaptive remodelling and recovery following eccentric exercise amongst a cohort of recreationally active males and females.

Chapter 4 demonstrated that the provision of ~20g protein and ~2g leucine supplementation designed to nutritionally target post exercise recovery from a predominantly animal derived protein accelerated the recovery of muscle function compared to a carbohydrate placebo. Due to increasing ethical and environmental concerns, there is an increasing presence and a growing research

field into sustainable plant-based sources. **Chapter 5** is the first study to utilise a plant-based protein supplement to nutritionally target post eccentric exercise recovery. However, contrary to our hypothesis the utilisation of a plant-based protein supplement, *Pisum Sativum*, providing 24.8g protein and 2.2g leucine, did not attenuate the decline in muscle function compared to an isocaloric placebo. Within **chapter 4**, the predominant source of protein utilised in the pooled data set was from animal derived protein sources, whey and casein protein. This accelerated the rate of recovery across both males and females compared to a carbohydrate placebo, aligning with the findings from other published work utilising milk protein [77], whey protein hydrolysate [188], whey and casein blend [127] which all accelerated the recovery of muscle function following eccentric exercise. Findings from Jackman, Witard [49] suggest that all amino acids may be required for eccentric exercise recovery, with muscle function not improving following the consumption of branched chain amino acids only. This highlights that there is a functional property within milk or animal derived protein sources that may have a protective mechanism or preventative mechanism that assists with recovery or prevents further aberrant damage happening at the site of muscle injury. Following eccentric exercise, it is well characterised that there is an inflammatory cascade of events due to the infiltration of neutrophils, macrophages and production of pro inflammatory cytokines in the systemic circulation and muscle tissues [342]. As suggested in **chapter 5**, milk derived proteins may have bioactive peptides which have anti-inflammatory properties [346], that have been shown to reduce the presence of pro-inflammatory cytokines and down regulate gene expression associated with inflammatory pathways such as NF-kB activation [127, 186]. This has been suggested to attenuate aberrant damage at the site of muscle injury caused by muscle protein

degradation and preserve muscle function. Findings from **chapter 6** demonstrate that the plant-based protein supplement has no influence over pro- or anti-inflammatory cytokines measured in the acute hours following eccentric exercise. Due to no study to date comparing plant based and animal derived protein sources ability to accelerate recovery following eccentric exercise, we provide suggestions that the functional properties of pea protein do not support accelerated recovery of muscle function however this does not discount pea protein having anti-inflammatory properties.

7.3 Muscle protein turnover during exercise recovery

Skeletal muscle undergoes cyclical changes of muscle protein turnover and is referred to as a plastic tissue, such that it can adapt and remodel to changing states of rest, exercise and unloading (immobilisation). Given that eccentric exercise induces myofibrillar disruption [28] and a greater myofibrillar protein synthesis rates compared to concentric exercise contractions [144], a greater availability of amino acids was hypothesised to enhance the myofibrillar protein synthetic response and assist with adaptive remodelling and recovery. There has been a limited range of studies investigating specific direct mechanisms associated with exercise induced muscle damage and muscle protein metabolism.

To determine free living integrative muscle protein synthesis over the time course of recovery, a deuterated water dosing strategy was utilised across **chapter 4-6** and presented in **chapter 2**. This deuterated water (deuterium oxide) dosing strategy was based on the body water pool contributing 60-70% body mass in healthy lean individuals [256] and the body water pool turning over at a rate of 9%·d^{-1} [115]. We were able to maintain a steady 0.6% body water ^{2}H labelling

over the measurement period to allow the sensitive determination of myofibrillar fractional synthetic rates over 3h (**Chapter 6**) and 1-3days (**Chapter 4** and **5**) of post eccentric exercise recovery.

In **Chapter 4**, MPS rates were determined over 1-3 days (24-72h) after a bout of eccentric exercise, this was due to previous observations of the biggest reductions in muscle function occurring around this time frame, such that the loss in muscle function is greatest and the rate of recovery of muscle function increases over this time period. These reductions in muscle function are suggestive of a key window whereby myofibrillar remodelling may be crucial for post eccentric exercise recovery. The data presented in **Chapter 4** demonstrate an accelerated recovery of muscle function alongside the ingestion of a protein supplement within the initial 24-48 h. This accelerated recovery leads to questions into the underlying mechanisms to post eccentric exercise recovery. The rates of daily myofibrillar protein synthesis were higher than previously reported amongst young individuals, when utilising a deuterated water dosing protocol. In previously published work reported rates under fasting conditions post exercise have been 1.93%·d^{-1} and 1.48%·d^{-1} [115] and 1.98%·d^{-1} and 1.64%·d^{-1} [116] across the ECC and CON leg respectively. Within **chapter 4** we present similar rates in the placebo condition of 2.11%·d^{-1} and 1.87 %·d^{-1} across the ECC and CON leg, respectively. However with the protein ingestion and repeated concentric exercise bouts, the supplementation condition trended to be higher, with daily FSR rates being 3.16%·d^{-1} and 2.16%·d^{-1} across the ECC and CON leg, respectively. We therefore express a larger anabolic response following eccentric exercise and two additional bouts of maximal concentric exercise (at 24 and 48h) than previously reported [115, 116] with trends for a greater influence

from the exogenous provision of amino acids across both the eccentrically exercised and contralateral control leg.

However within **chapter 4,** muscle function in males had recovered by 24h. This was suggestive that the majority of the myofibrillar repair and remodelling would have occurred within an earlier time frame. This then led to **Chapter 5,** investigating whether a protein supplement increases myofibrillar protein synthesis rates during the initial 24 and 48h period and if this influenced the rapid rate of recovery over this time frame. However within **chapter 5**, the plant-based protein supplement did not accelerate the recovery of muscle function compared to a placebo, however we are certain that adequate substrate was provided with the postprandial aminoacidemia, presented in **Figure 6.3**. With the biggest reductions in muscle function occurring between 24 and 48h. Within **chapter 5**, we demonstrated that MPS rates were no different between protein and placebo supplementation over 24 h following eccentric exercise, with large fractional synthetic rates across both ECC and CON legs. However we demonstrated that over 0-48 h of recovery MPS rates are significantly greater with the provision of a protein supplement, which aligns with data presented by Pavis, Jameson [78] whereby beyond 72 h of post eccentric exercise recovery the MPS rates begin to decline without the additional provision of exogenous amino acids. This presents a critical time period in which the provision of protein may assist with repair and remodelling, demonstrated by an accelerated rate of post exercise recovery beyond 48h in **Chapter 5**.

Findings from **chapter 5**, point towards an acceleration in the rate of muscle protein breakdown following eccentric exercise, providing endogenous amino acids to support the elevated MPS rates in the initial 24 h following exercise. Such

that there were no differences between carbohydrate and protein feeding across both the ECC and CON legs. Due to the large anabolic responses, this is suggestive that muscle protein breakdown is providing substrate and supporting elevated synthetic pathways, albeit maintaining a negative protein balance and delaying post eccentric exercise recovery. The demand for amino acids and the potential utilisation of endogenous amino acids was shown in **chapter 6**, whereby amino acids in the systemic circulation declined in the immediate hours following exercise, highlighting the demand for substrates for muscle remodelling and the high rates of MPS across both legs. Beyond 48h, whereby we show a greater MPS rate with the continued provision of exogenous amino acids and an acceleration in the rate of muscle functional recovery, this is the time frame where muscle protein synthesis [78] and breakdown rates [88] decline. Indicating that the continued provision within this key anabolic time frame following eccentric exercise is beneficial to supporting post eccentric exercise recovery and muscle remodelling.

The time course of integrative MPS responses utilising a deuterated water dosing protocols pooled from studies completed within the Nutritional Physiology research group is presented in **Figure 7.1**, encompassing a range of states whereby muscle protein turnover and the demand for amino acids differ. This graphical depiction demonstrates the additive benefit of protein above exercise alone in both concentric and eccentric exercise conditions. Whereby in the acute hours post eccentric exercise we see a maximised response to 20g protein and 2g leucine. This heightened MPS response is maintained over 24 and 48h, with the provision of protein and returns to basal values within 72h. During periods of injury, which may coincide with periods of immobilisation (IMMOB), this alters the

muscle protein balance, demonstrated to be due to a decline in basal myofibrillar protein synthesis rates and developing a resistance to the anabolic properties of protein [359, 360]. With the addition of an eccentric exercise stimulus (ECC-IMMOB) this can attenuate these declines in MPS following immobilisation [133]. These are all states which induce both anabolic and catabolic conditions associated with muscle damage and inflammation. The thought of proteolysis or catabolism is negative as it is associated with muscle atrophy and leading to functional weakness. However, the body is in a constant state of protein turnover which is a necessity for properly functioning skeletal muscle tissue. Following immobilisation the increased expression of ubiquitin proteasome pathway has been evidenced [359]. Resistance exercise has upregulated MuRF1 and MAFbx, suggesting an increased proteasome mediated proteolysis post resistance exercise [124, 130]. These markers indicate an increase in proteolytic pathways however are static molecular markers and as such should not be taken as conclusive indicators of muscle protein breakdown. After exercise in the post absorptive state, muscle net balance is negative [94], hyperaminoacidemia from the ingestion of amino acids is effective at switching the balance to positive and maximising the anabolic effect of exercise [124]. However in the circumstance of injury or damage does the provision of amino acids spare skeletal muscle.

Within **chapter 4**, we present that recovery occurred within 24 and 48 h following eccentric exercise suggestive that the repair and remodelling process is accelerated. Which may indicate that the provision of exogenous amino acids may downregulate inflammatory and proteolytic pathways, spare skeletal muscle protein degradation and as such prevent the breakdown of contractile proteins, preserving muscle function and return to muscle homeostasis quicker. The preservation of muscle function was observed within **Chapter 6**, due to a trend

for an increased muscle protein turnover. However this was not sustained due to suggestions that the plant-based proteins may not have downregulated inflammatory pathways and subsequent degradation over the acute day recovery window.

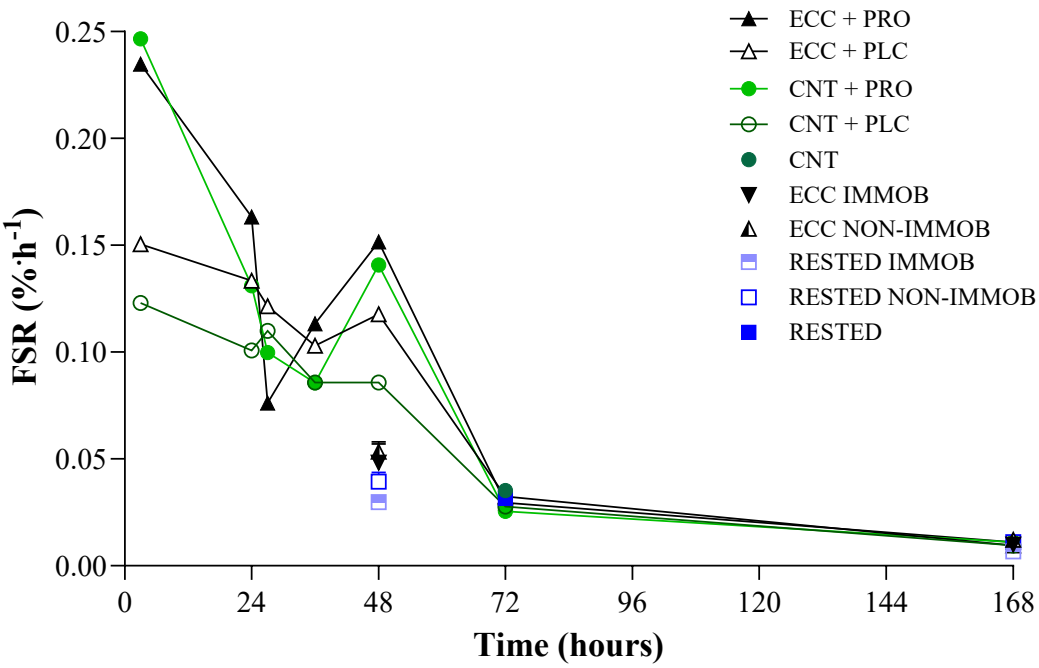

Figure 7.1 *Time course of muscle protein synthetic responses, expressed as fractional synthetic rate (FSR) over hours following bouts of eccentric (ECC) and concentric (CNT), limb immobilisation (IMMOB), rested (no exercise or immobilisation) and with either protein (PRO) or carbohydrate placebo (PLC) consumption.*

7.4 Adaptive remodelling

The concept of adaptation or remodelling implies an improvement in condition, in this case muscle proteins. This is evident in the protective mechanism termed the repeated bout effect, where the recurrence of the same exercise reduces the extent of muscle disruption [43, 62]. Similarly, hypertrophic gains in muscle mass occur once the muscle has adapted, as evidenced by a decreased presence of

skeletal muscle damage markers and a reduction in the MPS response following repeated exercise bouts [41].

In the context of hypertrophic adaptations, mechanical loading prompts radial muscle growth, increasing sarcomeres in parallel and an increase in muscle cross-sectional area over time. However, the process of adapting and overcoming damage involves mechanical disruption to the skeletal muscle, leading to positive effects such as building sarcomeres in series [35, 361]. Longitudinal muscle sectioning after eccentric exercise reveals an increased number of sarcomeres in series, resulting in longer fascicle lengths [362]. This adaptation reduces the length change of sarcomeres during subsequent eccentric contractions, such that the velocities and length changes are additive and the stress is shared, contributing to enhanced muscle integrity [362].

Satellite cells within the myofiber act as progenitors for self-repair, crucial for muscle regeneration after injury. Interestingly, myofiber self-repair can occur independently of satellite cells within 96 hours after exercise [363]. Increased myonuclear migration and expression, detected 24 h after injury, coincide with the detection of sarcomere repair proteins and realignment of sarcomere constituents [363]. A sufficient provision of amino acids at the site of injury, was alluded to have a protective effect on subsequent damage inducing processes such that it provides sufficient substrate for muscle remodelling. The source of these amino acids is uncertain. Skeletal muscle is a reservoir of amino acids, approximately 40% of amino acids for *de novo* protein synthesis come from endogenous proteins at rest, with an increased release after injury or illness in support of returning to a state of homeostasis [119]. Previously published work has demonstrated that exercise induces an increased intramuscular ‘recycling’ of

amino acids from the breakdown of muscle protein, with rates increasing from basal by 16-18% [88, 129]. These amino acids can be repartitioned and enter the intracellular pool to stimulate protein synthesis through posttranscriptional mechanisms. This is demonstrated and alluded to be why MPS rates are elevated under placebo conditions within this thesis, such that there is no clear statistical difference between protein feeding and placebo conditions.

The noteworthy aspect is that the adaptive response is integral to the recovery process, facilitating skeletal muscle adaptation. However, by strategically targeting post-exercise recovery through protein nutrition, there's a potential alteration of this process. Nutritional interventions, particularly exogenous amino acid provision, may suppress muscle protein breakdown, hindering the natural adaptive response. This could involve down-regulating muscle protein degradation, creating conditions conducive to an increase in the rate of muscle protein synthesis. However, within **chapter 4**, we present that muscle functional ability is restored within 24-48 h, such that within this acute time window post eccentric exercise, nutritionally targeting recovery has a protective effect. It has been demonstrated that the provision of animal derived protein reduces inflammatory pathways associated with muscle protein degradation [127, 186] and thus may prevent the reduction in muscle function, through retaining the integrity of the muscle. However, we have yet to determine whether the provision of protein, reduces the extent of muscle protein breakdown or makes muscle protein net balance less negative following eccentric exercise. We were not able to determine the muscle protein breakdown rates within this thesis and as such propose a study design to which rates of muscle protein turnover 24 h following eccentric exercise could be determined.

7.5 Implications and future direction

Within **chapter 3**, we provided further evidence of an age associated decline in sensitivity to anabolic stimuli such that the there was a dose response between leucine dose and post exercise MPS response in older adults. Such that leucine becomes of more relevance in terms of governing post-exercise postprandial muscle protein synthesis rates in senescent muscle. Older adults are recommended to do resistance training and increase protein intake to support the maintenance of muscle mass and a vast majority of research focuses on maximising the anabolic response. However muscle function (muscle strength) is overlooked in relation to supporting physical independence in older individuals. It is assumed that with a greater muscle mass there is a sufficient muscle function, however this may not always be the case. Older adults with the lowest muscle function are at an increased risk of losing independence compared to those with a lower muscle mass [364]. Low muscle mass and low muscle function are both prerequisites for sarcopenia however they can occur independently of each other [364]. They can also be more pronounced following periods of disuse/ bed rest and illness. Within this thesis we demonstrate that eccentric exercise increases the MPS response to a greater extent than resistance exercise studies. Further, eccentric exercise training within the elderly population has demonstrated a 20% increase of sarcomeres in series compared to concentric resistance training [365]. This increase in sarcomeres in series can assist with force generating capacity and physical function. As such future research could determine the efficacy of utilising a protein supplement to offset the negative side effects of eccentric exercise (soreness) but promote muscle remodelling amongst older adults.

Due to potential sex differences in physiology, females have been underrepresented in human research, potentially leading to inappropriate recommendations when findings from experimental research involving only one sex are extrapolated to both sexes [366]. The variability in sex hormones across the menstrual cycle requires standardised methods to determine these fluctuations such as urinary luteinizing hormone (LH) measurement to predict ovulation, typically occurring within 14-26 hours after the LH surge. Other methods include basal body temperature and salivary hormone analysis, with serum hormone analysis being considered the gold standard despite its increased burden and costs [336]. In **Chapter 4**, the study was not statistically powered to detect sex differences, and menstrual cycle phase was determined using a calendar-based counting method to approximate the follicular phase when sex hormones are lowest. This criterion and viewpoint are suggestive of hormonal fluctuations being a confounding factor. However, the narrative should be adjusted to explore the physiological implications of these hormonal fluctuations. **Chapter 4** revealed distinct recovery profiles following eccentric exercise for males and females, suggesting potential differences in protein nutrition and supplementation needs following strenuous exercise. This underscores the importance of considering sex-specific responses in future research and in designing exercise and nutrition interventions.

7.5.1 Characterising muscle protein breakdown following eccentric exercise

As alluded to across the experimental chapters throughout this thesis, muscle protein breakdown is an underexplored physiological pathway which has potential implications towards exercise recovery after damaging exercise. No study has measured muscle protein breakdown (MPB) during recovery from

eccentric exercise alongside the ingestion of a plant-based protein supplement. This proposed study will provide novel information characterising rates of muscle protein breakdown 24 h after eccentric exercise. This will assist in determining if the rate of MPB underpins the large MPS responses seen throughout this thesis. As well as determining whether the exogenous provision of amino acids suppresses MPB by sparing skeletal muscle from degradation, assist with preventing further losses in muscle function and lead to a more positive net protein balance.

Figure 7.2 presents some hypothetical results based on the fractional synthetic rate (FSR) data at 24h drawn from **Chapter 5** and fractional breakdown rate (FBR) within the rested control leg at 24h being 0.1%/h^{-1} [139]. Alongside a subsequent increase in FBR following eccentric exercise. Based on severe burns patients proteolysis was 40% greater than control patients [367]. Following resistance exercise, proteolysis was 18% greater than basal FBR [88]. Following damaging exercise, we expect there to be a slightly greater FBR response than resistance exercise due to the structural damage to the contractile machinery of the muscle and increased inflammatory response and associated degradation. Net protein balance would thus be negative over the 24h period however would be less negative in the protein nutrition condition (VGP) compared to the PLC condition and have a sparing effect on overall muscle protein turnover, elevating MPS rates but ultimately reducing the extent of muscle protein breakdown.

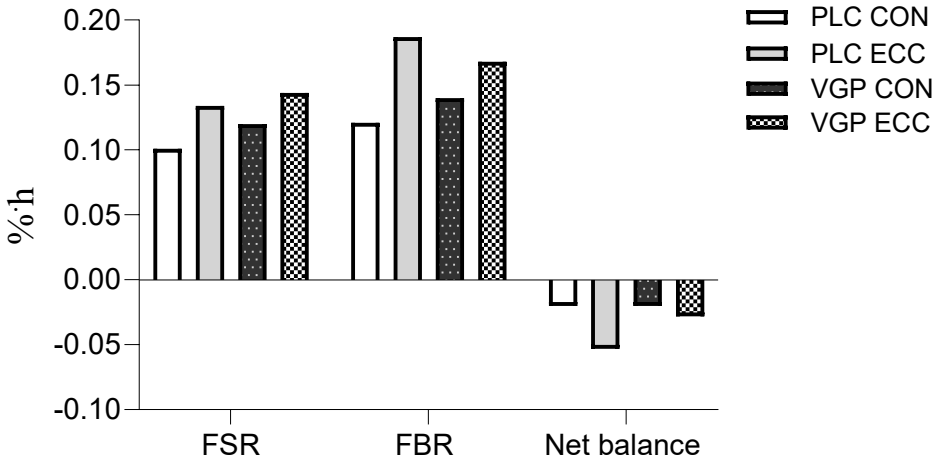

Figure 7.2 *Hypothetical data from the proposed study design to determine muscle protein net balance following eccentric exercise utilising measures of fractional synthetic rate (FSR) and fractional breakdown rate (FBR) of mixed muscle proteins*

7.5.2 Study design

Utilising the same participant recruitment characterisation and eccentric exercise protocol as presented in **Chapter 2** and utilised throughout this thesis. Following screening and inclusion into the study, participants would be randomly assigned to either the protein supplementation or the placebo supplementation condition in a double blinded placebo controlled parallel study design. For 7 days prior to eccentric exercise participants would consume the protein or placebo supplement once daily and be asked to maintain habitual diet and exercise habits throughout the duration of the study. A schematic overview of the study protocol is presented in **Figure 7.3**. Exactly 24h before the start of the experimental test day, participants will arrive overnight fasted for a baseline blood sample, muscle function test (**Section 2.3.4** and **2.3.5**) and complete the unilateral damaging eccentric exercise protocol (**Section 2.3.3**) before consuming the post exercise supplement. The leg randomised to undergo eccentric exercise will be

counterbalanced within each group for leg dominance, while the contralateral leg will serve as the within subject control leg. Diet will be controlled for the 24 h proceeding the experimental test day. Energy requirements will be calculated using the Henry equation [253] multiplied by an activity factor of 1.6. Protein intake will be controlled at 1.2g·kg body weight^{-1}day^{-1} (**Section 2.4.3**). Participants will be instructed to abstain from any caffeine, alcohol, anti-inflammatory medications, or strenuous physical activity for the duration of the study.

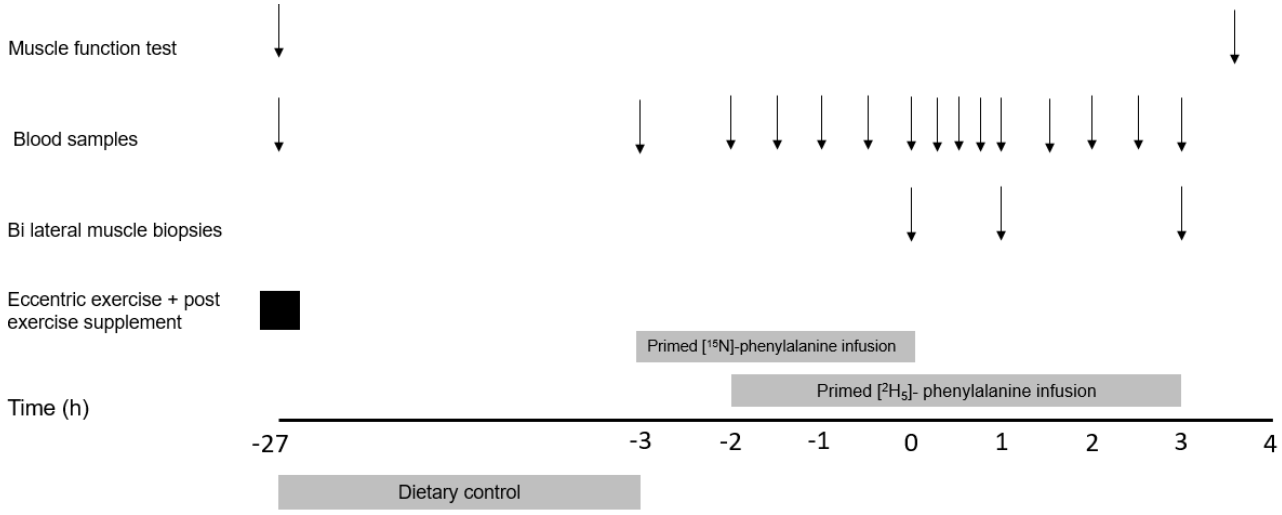

Figure 7.3 *Schematic representation of the study outline. Dietary control and supplementation for 24hours. Single day stable isotope infusion will commence 24hours after damaging exercise for the quantification of muscle protein synthesis and breakdown. Eccentric exercise + post exercise supplement: 300 maximal unilateral eccentric contractions of the knee extensors followed by consuming a vegan post exercise supplement or an isocaloric placebo. Biopsies: vastus lateralis bilateral (exercised and control leg) muscle biopsies. Blood samples: arterialised blood sample collection. Muscle function: unilateral maximal isometric and isokinetic concentric contractions of the quadriceps measured by isokinetic dynamometry. Exercised and control leg completed all function tests.*

After an overnight fast and 24 h after eccentric exercise, participants will return to the laboratory. A Teflon canula will be inserted into the antecubital vein of one arm for the infusion of the stable isotope tracer. Before the initiation of the infusion, a baseline venous blood sample will be taken from this site to measure the background isotopic enrichments. The infusion protocol will begin with a single intravenous priming dose of L-[^{15}N]-phenylalanine. After the priming dose, a continuous infusion will be initiated. Repeated arterialised venous blood samples will be obtained from a heated (55°C) dorsal hand vein and kept patent with a continuous 0.9% saline infusion. Sixty minutes into the L-[^{15}N]-phenylalanine infusion, a primed-continuous infusion of L-[ring-$^{2}H_5$]phenylalanine will begin and continue for the remainder of the experimental trial. At time (t) 0, bilateral biopsies of the *m. vastus lateralis* will be obtained under local anaesthesia (2% lidocaine). The L-[^{15}N]-phenylalanine infusion will then immediately be stopped. Additional muscle biopsies will be obtained at t = 60 and 180 min, following the cessation of the L-[^{15}N]-phenylalanine infusion. The biopsy samples will be ~2.5 cm proximal to the previous incision. Biopsy samples will be rapidly frozen in liquid nitrogen and stored at -80°C for further analysis. Arterialised venous blood samples will be taken throughout the remainder of the infusion at the following time points: 0, 15, 30, 45, 60, 90, 120, 150,180minutes. A further muscle function test will be performed following the final muscle biopsy.

Blood plasma analysis will be completed as stated in **Section 2.5.4.** Amino acids from intracellular and mixed muscle protein bound pools would be extracted utilising the methodological analysis from Pavis, Abdelrahman [139].

A modified precursor product equation will be used to calculate fractional breakdown rate (FBR) of mixed muscle proteins [138]. This equation utilises the

decay in arterial blood plasma and the muscle intracellular pool enrichments over the time period after the isotope tracer is stopped, such that the rate at which tracee is released from protein breakdown dilutes the intracellular enrichment. This can be combined with calculating FSR of mixed muscle proteins using the product precursor equation to determine muscle protein net balance (*FSR-FBR*).

7.5.3 Limitations

Utilising contemporary methodologies to investigate muscle protein synthesis rates over multiple days of recovery and a robust unilateral within subject contralateral control leg model to determine the influence of nutrition on skeletal muscle recovery following a bout of eccentric exercise is a strength of this thesis.

Another notable strength is the full dietary control throughout the experimental period. Markedly, among the studies examining recovery from eccentric exercise and protein supplementation, as outlined in **Table 1.1**, only three out of ten studies implemented this level of dietary control. While current recommended daily allowance (RDA) advocate for a protein intake of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$, there are no established RDA specifically tailored to exercise requirements. However, a suggested intake of 1.2$g \cdot kg^{-1} \cdot d^{-1}$ is recommended for individuals engaged in physical exercise, specifically resistance exercise [368, 369]. It's worth noting that individuals actively involved in exercise typically exceed this RDA, with participants in this thesis who engage in recreational activity habitually consuming around 1.2 $g \cdot kg^{-1} \cdot d^{-1}$. This level of intake is deemed safe and effective, particularly in the context of resistance exercise [368]. The inclusion of a protein supplement within the controlled diet elevated protein levels to 1.6 $g \cdot kg^{-1} \cdot d^{-1}$. However, **Chapter 5** demonstrated that this daily

increase in protein intake did not support recovery in comparison to providing 1.2g·kg^{-1}·d^{-1} of protein from diet alone. This prompts the consideration that the baseline protein intake may have overshadowed any potential benefits of the supplement. It appears that when adequate amino acids are readily available through daily dietary sources, the additional intake via supplementation may not confer added advantages. This suggests that muscles may reach a saturation point, wherein excess amino acids are not effectively utilised.

Chapters 5 and **6** utilised a carbohydrate placebo as a comparator to the plant based protein supplement, which we recognise as a limitation. It is important to note that these chapters were part of a broader clinical trial featuring three study arms: a plant-based protein supplement (pea protein), an animal-based protein supplement (whey protein), and the carbohydrate placebo. Due to industry funding the comparator for this thesis could only be the carbohydrate placebo and as such future work will utilise the animal based protein supplement as the comparator.

The utilisation of the isokinetic dynamometer in assessing eccentric exercise and muscle function provided controlled velocity and range of motion, ensuring consistency across repetitions. However, alternative methodologies, such as eccentric actions in resistance exercise or downhill running, may better simulate common exercise settings. Notably, greater reductions in muscle function are observed in the *biceps brachii* compared to the quadriceps muscle group [67], likely due to differences in pennation angles and habitual use of lower limbs, potentially affording some level of repeated bout effect protection in recreationally active individuals. Further investigation into the necessity of 300 eccentric contractions is warranted, as similar reductions to **chapters 4-6**

(~25% loss in muscle function) have been observed with only 100 eccentric contractions in sedentary individuals [59, 61]. This suggests that beyond 100 eccentric contractions, the induction of muscular fatigue rather than muscle damage may be predominant.

In **Chapter 6**, blood sampling time points postprandial were hourly, thus not capturing the entire postprandial amino acid response. While a more detailed exploration could have been pursued, the decision aimed to minimise participant burden, considering the repeated blood and muscle sampling alongside daily laboratory visits within the wider experimental trail. The primary focus remained on determining muscle protein synthetic response within this 3h timeframe.

7.6 Conclusions

The studies presented within this thesis have investigated for the first time the influence of a plant-based pea protein supplement on the recovery of muscle function in recreationally active males and females and has attempted to investigate the association between myofibrillar protein synthesis and eccentric exercise recovery. The studies were performed under controlled conditions within the Nutritional Physiology research unit at the University of Exeter. Utilising contemporary methodologies to investigate muscle protein synthesis rates over multiple days of recovery and a robust unilateral within subject contralateral control leg model to determine the influence of nutrition on skeletal muscle recovery following a bout of eccentric exercise. Further, the thesis included a systematic review to assess the influence of leucine on postprandial postexercise muscle protein synthesis rates.

We have demonstrated that postprandial postexercise MPS responses cannot be predicted from any single dose or plasma leucine variable and as such are not determined by the leucine trigger or leucine threshold hypothesis. The efficacy of a protein source designed to nutritionally target post eccentric exercise recovery was demonstrated. However, the use of a plant-based protein supplement providing ample protein and leucine did not support an accelerated recovery rate following eccentric exercise. This thesis did establish that MPS rates were increased over 3h following eccentric exercise and this was associated with a greater ability to produce more isokinetic work over this time frame. Further, the continued provision of protein and exercise stimuli over 48h post eccentric exercise supported elevated MPS rates and an accelerated rate of recovery. However, over 24h whereby it was shown that muscle function had recovered with protein supplementation, but MPS rates were high across both conditions, this was alluded to be due to an elevation in the endogenous provision of amino acids from muscle protein breakdown over 24h following exercise. It is therefore of interest for future interventions to understand the influence of muscle protein breakdown on driving losses in muscle function and changes to muscle protein turnover following eccentric exercise.

8 References

1. Frontera, W.R. and Ochala, J. 2015. Skeletal Muscle: A Brief Review of Structure and Function. *Calcified Tissue International,* 96**,** 183-195.
2. McKendry, J., Stokes, T., McLeod, J.C., and Phillips, S.M. 2021. Resistance Exercise, Aging, Disuse, and Muscle Protein Metabolism. *Comprehensive Physiology,* 11**,** 2249-2278.
3. Hawley, J.A., Hargreaves, M., Joyner, M.J., and Zierath, J.R. 2014. Integrative biology of exercise. *Cell,* 159**,** 738-49.
4. Ibata, N. and Terentjev, E.M. 2021. Why exercise builds muscles: Titin mechanosensing controls skeletal muscle growth under load. *arXiv preprint arXiv:2102.00836*.
5. Jorgenson, K.W., Phillips, S.M., and Hornberger, T.A. 2020. Identifying the Structural Adaptations that Drive the Mechanical Load-Induced Growth of Skeletal Muscle: A Scoping Review. *Cells,* 9**,** 1658.
6. Burgoyne, T., Morris, E.P., and Luther, P.K. 2015. Three-dimensional structure of vertebrate muscle Z-band: the small-square lattice Z-band in rat cardiac muscle. *Journal of molecular biology,* 427**,** 3527-3537.
7. Luther, P.K. 2009. The vertebrate muscle Z-disc: sarcomere anchor for structure and signalling. *Journal of Muscle Research and Cell Motility,* 30**,** 171-185.
8. Leonard, T.R. and Herzog, W. 2010. Regulation of muscle force in the absence of actin-myosin-based cross-bridge interaction. *American Journal of Physiology-Cell Physiology,* 299**,** C14-C20.
9. Horowits, R., Kempner, E.S., Bisher, M.E., and Podolsky, R.J. 1986. A physiological role for titin and nebulin in skeletal muscle. *Nature,* 323**,** 160-164.
10. Macaluso, F., Isaacs, A.W., Di Felice, V., and Myburgh, K.H. 2014. Acute change of titin at mid-sarcomere remains despite 8 wk of plyometric training. *J Appl Physiol (1985),* 116**,** 1512-9.
11. Féasson, L., Stockholm, D., Freyssenet, D., Richard, I., Duguez, S., Beckmann, J.S., and Denis, C. 2002. Molecular adaptations of neuromuscular disease-associated proteins in response to eccentric exercise in human skeletal muscle. *The Journal of Physiology,* 543**,** 297-306.
12. Rebbeck, R.T., Karunasekara, Y., Board, P.G., Beard, N.A., Casarotto, M.G., and Dulhunty, A.F. 2014. Skeletal muscle excitation–contraction coupling: who are the dancing partners? *The international journal of biochemistry & cell biology,* 48**,** 28-38.
13. Huxley, H. and Hanson, J. 1954. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature,* 173**,** 973-976.
14. Proske, U. and Morgan, D.L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of physiology,* 537**,** 333-345.
15. Bigland-Ritchie, B. and Woods, J. 1976. Integrated electromyogram and oxygen uptake during positive and negative work. *The Journal of physiology,* 260**,** 267-277.
16. Katz, B. 1939. The relation between force and speed in muscular contraction. *The Journal of physiology,* 96**,** 45.
17. Asmussen, E. 1953. Positive and Negative Muscular Work. *Acta Physiologica Scandinavica,* 28**,** 364-382.

18. Armstrong, R., Warren, G., and Warren, J. 1991. Mechanisms of exercise-induced muscle fibre injury. *Sports medicine,* 12**,** 184-207.

19. Peake, J.M., Suzuki, K., Wilson, G., Hordern, M., Nosaka, K., Mackinnon, L., and Coombes, J.S. 2005. Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation. *Medicine & Science in Sports & Exercise,* 37**,** 737-745.

20. Proske, U. and Allen, T.J. 2005. Damage to skeletal muscle from eccentric exercise. *Exercise and sport sciences reviews,* 33**,** 98-104.

21. Sorichter, S., Puschendorf, B., and Mair, J. 1999. Skeletal muscle injury induced by eccentric muscle action: muscle proteins as markers of muscle fiber injury. *Exercise immunology review,* 5**,** 5-21.

22. Morgan, D.L. and Allen, D. 1999. Early events in stretch-induced muscle damage. *Journal of Applied physiology,* 87**,** 2007-2015.

23. Lieber, R.L., Thornell, L.-E., and Fridén, J. 1996. Muscle cytoskeletal disruption occurs within the first 15 min of cyclic eccentric contraction. *Journal of applied physiology,* 80**,** 278-284.

24. Lieber, R.L. and Fridén, J. 2002. Mechanisms of muscle injury gleaned from animal models. *American journal of physical medicine & rehabilitation,* 81**,** S70-S79.

25. Paulsen, G., Ramer Mikkelsen, U., Raastad, T., and Peake, J.M. 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exercise immunology review,* 18.

26. Heffner, R. 1975. Electron microscopy of disorders of skeletal muscle. *Annals of Clinical & Laboratory Science,* 5**,** 338-347.

27. Knappeis, G. and Carlsen, F. 1962. The ultrastructure of the Z disc in skeletal muscle. *The Journal of cell biology,* 13**,** 323-335.

28. Friden, J., Sjöström, M., and Ekblom, B. 1983. Myofibrillar damage following intense eccentric exercise in man. *International journal of sports medicine,* 4**,** 170-176.

29. Friden, J., Sjöström, M., and Ekblom, B. 1981. A morphological study of delayed muscle soreness. *Experientia,* 37**,** 506-507.

30. Newham, D.J., McPhail, G., Mills, K.R., and Edwards, R.H.T. 1983. Ultrastructural changes after concentric and eccentric contractions of human muscle. *Journal of the Neurological Sciences,* 61**,** 109-122.

31. Raastad, T., Owe, S.G., Paulsen, G., Enns, D., Overgaard, K., Crameri, R., Kiil, S., Belcastro, A., Bergersen, L., and Hallén, J. 2009. Changes in calpain activity, muscle structure, and function after eccentric exercise. *Medicine & Science in Sports & Exercise,* 42**,** 86-95.

32. Gibala, M., MacDougall, J., Tarnopolsky, M., Stauber, W., and Elorriaga, A. 1995. Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. *Journal of Applied Physiology,* 78**,** 702-708.

33. Beaton, L.J., Tarnopolsky, M.A., and Phillips, S.M. 2002. Contraction-induced muscle damage in humans following calcium channel blocker administration. *The Journal of physiology,* 544**,** 849-859.

34. Crameri, R., Aagaard, P., Qvortrup, K., Langberg, H., Olesen, J., and Kjær, M. 2007. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *The Journal of physiology,* 583**,** 365-380.

35. Yu, J.-G., Carlsson, L., and Thornell, L.-E. 2004. Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochemistry and cell biology,* 121**,** 219-227.

36. Mair, J., Koller, A., Artner-Dworzak, E., Haid, C., Wicke, K., Judmaier, W., and Puschendorf, B. 1992. Effects of exercise on plasma myosin heavy chain fragments and MRI of skeletal muscle. *Journal of Applied Physiology,* 72**,** 656-663.

37. MacIntyre, D.L., Sorichter, S., Mair, J., Berg, A., and McKenzie, D.C. 2001. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *European journal of applied physiology,* 84**,** 180-186.

38. Carlsson, L. and Thornell, L.E. 2001. Desmin-related myopathies in mice and man. *Acta Physiologica Scandinavica,* 171**,** 341-348.

39. Yu, J.G., Malm, C., and Thornell, L.E. 2002. Eccentric contractions leading to DOMS do not cause loss of desmin nor fibre necrosis in human muscle. *Histochem Cell Biol,* 118**,** 29-34.

40. Yu, J.G. and Thornell, L.E. 2002. Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study. *Histochem Cell Biol,* 118**,** 171-9.

41. Damas, F., Phillips, S.M., Libardi, C.A., Vechin, F.C., Lixandrão, M.E., Jannig, P.R., Costa, L.A., Bacurau, A.V., Snijders, T., and Parise, G. 2016. Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage. *The Journal of Physiology,* 594**,** 5209-5222.

42. Gibala, M.J., Interisano, S.A., Tarnopolsky, M.A., Roy, B.D., MacDonald, J.R., Yarasheski, K.E., and MacDougall, J.D. 2000. Myofibrillar disruption following acute concentric and eccentric resistance exercise in strength-trained men. *Canadian Journal of Physiology and Pharmacology,* 78**,** 656-661.

43. Hyldahl, R.D., Chen, T.C., and Nosaka, K. 2017. Mechanisms and mediators of the skeletal muscle repeated bout effect. *Exercise and sport sciences reviews,* 45**,** 24-33.

44. Stauber, W., Clarkson, P., Fritz, V., and Evans, W. 1990. Extracellular matrix disruption and pain after eccentric muscle action. *Journal of applied physiology,* 69**,** 868-874.

45. Hyldahl, R.D. and Hubal, M.J. 2014. Lengthening our perspective: morphological, cellular, and molecular responses to eccentric exercise. *Muscle & Nerve,* 49**,** 155-170.

46. Peake, J.M., Neubauer, O., Della Gatta, P.A., and Nosaka, K. 2017. Muscle damage and inflammation during recovery from exercise. *Journal of applied physiology,* 122**,** 559-570.

47. Belcastro, A.N., Shewchuk, L.D., and Raj, D.A. 1998. Exercise-induced muscle injury: a calpain hypothesis. *Molecular and cellular biochemistry,* 179**,** 135-145.

48. Warren, G.L., Ingalls, C.P., Lowe, D.A., and Armstrong, R. 2001. Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exercise and sport sciences reviews,* 29**,** 82-87.

49. Jackman, S.R., Witard, O.C., Jeukendrup, A.E., and Tipton, K.D. 2010. Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. *Medicine and science in sports and exercise,* 42**,** 962-970.

50. Nosaka, K. and Clarkson, P. 1996. Variability in Serum Creatine Kinase Response After Eccentric Exercise of the Elbow Flexors. *International Journal of Sports Medicine,* 17**,** 120-127.

51. Clarkson, P.M. and Ebbeling, C. 1988. Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clinical Science (London, England: 1979),* 75**,** 257-261.

52. Peake, J., Neubauer, O., Della Gatta, P.A., and Nosaka, K. 2017. Muscle damage and inflammation during recovery from exercise. *Journal of applied physiology,* 122**,** 559-570.

53. Chazaud, B., Sonnet, C., Lafuste, P., Bassez, G., Rimaniol, A.-C., Poron, F., Authier, F.-J., Dreyfus, P.A., and Gherardi, R.K. 2003. Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. *The Journal of cell biology,* 163**,** 1133-1143.

54. Deng, B., Wehling-Henricks, M., Villalta, S.A., Wang, Y., and Tidball, J.G. 2012. IL-10 triggers changes in macrophage phenotype that promote muscle growth and regeneration. *The Journal of Immunology,* 189**,** 3669-3680.

55. Lowe, D.A., Warren, G.L., Ingalls, C.P., Boorstein, D.B., and Armstrong, R. 1995. Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *Journal of applied physiology,* 79**,** 1260-1270.

56. Mikkelsen, U.R., Langberg, H., Helmark, I.C., Skovgaard, D., Andersen, L.L., Kjaer, M., and Mackey, A.L. 2009. Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. *Journal of applied physiology,* 107**,** 1600-1611.

57. Paschalis, V., Koutedakis, Y., Jamurtas, A.Z., Mougios, V., and Baltzopoulos, V. 2005. Equal volumes of high and low intensity of eccentric exercise in relation to muscle damage and performance. *The Journal of Strength & Conditioning Research,* 19**,** 184-188.

58. Armstrong, R., Ogilvie, R., and Schwane, J. 1983. Eccentric exercise-induced injury to rat skeletal muscle. *Journal of Applied Physiology,* 54**,** 80-93.

59. Buckley, J.D., Thomson, R.L., Coates, A.M., Howe, P.R., DeNichilo, M.O., and Rowney, M.K. 2010. Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *Journal of Science and Medicine in Sport,* 13**,** 178-181.

60. White, J.P., Wilson, J.M., Austin, K.G., Greer, B.K., St John, N., and Panton, L.B. 2008. Effect of carbohydrate-protein supplement timing on acute exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition,* 5**,** 1-7.

61. Ives, S.J., Bloom, S., Matias, A., Morrow, N., Martins, N., Roh, Y., Ebenstein, D., O'Brien, G., Escudero, D., Brito, K., Glickman, L., Connelly, S., and Arciero, P.J. 2017. Effects of a combined protein and antioxidant supplement on recovery of muscle function and soreness following eccentric exercise. *J Int Soc Sports Nutr,* 14**,** 21.

62. Nosaka, K.K. 2008. Muscle soreness and damage and the repeated-bout effect.

63. Nosaka, K., Newton, M., and Sacco, P. 2002. Responses of human elbow flexor muscles to electrically stimulated forced lengthening exercise. *Acta Physiologica Scandinavica,* 174**,** 137-145.

64. Nuzzo, J.L., Pinto, M.D., and Nosaka, K. 2023. Overview of muscle fatigue differences between maximal eccentric and concentric resistance exercise. *Scandinavian Journal of Medicine & Science in Sports*.

65. Newham, D., Jones, D., Ghosh, G., and Aurora, P. 1988. Muscle fatigue and pain after eccentric contractions at long and short length. *Clinical Science,* 74**,** 553-557.

66. Clarkson, P.M., Nosaka, K., and Braun, B. 1992. Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and science in sports and exercise,* 24**,** 512-520.

67. Cleak, M.J. and Eston, R.G. 1992. Delayed onset muscle soreness: Mechanisms and management. *Journal of Sports Sciences,* 10**,** 325-341.

68. Newham, D., Jones, D., and Edwards, R. 1983. Large delayed plasma creatine kinase changes after stepping exercise. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine,* 6**,** 380-385.

69. Pyne, D.B. 1994. Exercise-induced muscle damage and inflammation: a review. *Australian journal of science and medicine in sport,* 26**,** 49-49.

70. Friden, J. 1984. Muscle soreness after exercise: implications of morphological changes. *International journal of sports medicine,* 5**,** 57-66.

71. Friden, J., Sfakianos, P., and Hargens, A. 1986. Muscle soreness and intramuscular fluid pressure: comparison between eccentric and concentric load. *Journal of Applied Physiology,* 61**,** 2175-2179.

72. Evans, G., Haller, R.G., Wyrick, P.S., Parkey, R.W., and Fleckenstein, J.L. 1998. Submaximal delayed-onset muscle soreness: correlations between MR imaging findings and clinical measures. *Radiology,* 208**,** 815-820.

73. Nosaka, K., Newton, M., and Sacco, P. 2002. Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scandinavian Journal of Medicine & Science in Sports,* 12**,** 337-346.

74. Asmussen, E. 1956. Observations on experimental muscular soreness. *Acta Rheumatologica Scandinavica,* 2**,** 109-116.

75. Abraham, W.M. 1977. Factors in delayed muscle soreness. *Medicine and science in sports,* 9**,** 11-20.

76. Armstrong, R. 1984. Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and science in sports and exercise,* 16**,** 529-538.

77. Cockburn, E., Hayes, P.R., French, D.N., Stevenson, E., and St Clair Gibson, A. 2008. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Appl Physiol Nutr Metab,* 33**,** 775-83.

78. Pavis, G.F., Jameson, T.S., Dirks, M.L., Lee, B.P., Abdelrahman, D.R., Murton, A.J., Porter, C., Alamdari, N., Mikus, C.R., and Wall, B.T. 2020. Improved recovery from skeletal muscle damage is largely unexplained by myofibrillar protein synthesis or inflammatory and regenerative gene expression pathways. *American Journal of Physiology-Endocrinology and Metabolism*.

79. Roig, M., O'Brien, K., Kirk, G., Murray, R., McKinnon, P., Shadgan, B., and Reid, W.D. 2009. The effects of eccentric versus concentric resistance training on muscle strength and mass in healthy adults: a systematic review with meta-analysis. *Br J Sports Med,* 43**,** 556-68.

80. Fridén, J. 1984. Changes in human skeletal muscle induced by long-term eccentric exercise. *Cell and tissue research,* 236**,** 365-372.

81. Fridén, J., Seger, J., Sjöström, M., and Ekblom, B. 1983. Adaptive Response in Human Skeletal Muscle Subjected to Prolonged Eccentric Training. *International Journal of Sports Medicine,* 04**,** 177-183.

82. Wall, B.T., Gorissen, S.H., Pennings, B., Koopman, R., Groen, B.B.L., Verdijk, L.B., and Van Loon, L.J.C. 2015. Aging Is Accompanied by a Blunted Muscle Protein Synthetic Response to Protein Ingestion. *PLOS ONE,* 10**,** e0140903.

83. Kilroe, S.P., Fulford, J., Jackman, S.R., LJC, V.A.N.L., and Wall, B.T. 2020. Temporal Muscle-specific Disuse Atrophy during One Week of Leg Immobilization. *Med Sci Sports Exerc,* 52**,** 944-954.

84. Dirks, M.L., Wall, B.T., Nilwik, R., Weerts, D.H., Verdijk, L.B., and van Loon, L.J. 2014. Skeletal muscle disuse atrophy is not attenuated by dietary protein supplementation in healthy older men. *The Journal of nutrition,* 144**,** 1196-1203.

85. Kobak, K.A., Lawrence, M.M., Pharaoh, G., Borowik, A.K., Peelor III, F.F., Shipman, P.D., Griffin, T.M., Van Remmen, H., and Miller, B.F. 2021. Determining the contributions of protein synthesis and breakdown to muscle atrophy requires non-steady-state equations. *Journal of Cachexia, Sarcopenia and Muscle*.

86. Wilkinson, D.J., Piasecki, M., and Atherton, P.J. 2018. The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. *Ageing Research Reviews,* 47**,** 123-132.

87. Biolo, G., Maggi, S.P., Williams, B.D., Tipton, K.D., and Wolfe, R.R. 1995. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *American Journal of Physiology-Endocrinology And Metabolism,* 268**,** E514-E520.

88. Phillips, S.M., Tipton, K.D., Aarsland, A., Wolf, S.E., and Wolfe, R.R. 1997. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of Physiology,* 273**,** E99-107.

89. Chesley, A., MacDougall, J., Tarnopolsky, M., Atkinson, S., and Smith, K. 1992. Changes in human muscle protein synthesis after resistance exercise. *Journal of applied physiology,* 73**,** 1383-1388.

90. Bohé, J., Low, A., Wolfe, R.R., and Rennie, M.J. 2003. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *The Journal of Physiology,* 552**,** 315-324.

91. Fujita, S., Dreyer, H.C., Drummond, M.J., Glynn, E.L., Cadenas, J.G., Yoshizawa, F., Volpi, E., and Rasmussen, B.B. 2007. Nutrient signalling in the regulation of human muscle protein synthesis. *The Journal of physiology,* 582**,** 813-823.

92. Tipton, K., Gurkin, B., Matin, S., and Wolfe, R. 1999. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *The Journal of nutritional biochemistry,* 10**,** 89-95.

93. Biolo, G., Tipton, K.D., Klein, S., and Wolfe, R.R. 1997. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *American Journal of Physiology-Endocrinology And Metabolism,* 273**,** E122-E129.

94. Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle, D., Jr., and Wolfe, R.R. 1999. Postexercise net protein synthesis in human muscle from orally administered amino acids. *American Journal of Physiology,* 276**,** E628-34.

95. Rasmussen, B.B., Tipton, K.D., Miller, S.L., Wolf, S.E., and Wolfe, R.R. 2000. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *Journal of Applied Physiology,* 88**,** 386-92.

96. Børsheim, E., Tipton, K.D., Wolf, S.E., and Wolfe, R.R. 2002. Essential amino acids and muscle protein recovery from resistance exercise. *American Journal of Physiology-Endocrinology and Metabolism*.

97. Laplante, M. and Sabatini, D.M. 2009. mTOR signaling at a glance. *Journal of cell science,* 122**,** 3589-3594.

98. Monteyne, A.J., Coelho, M.O., Porter, C., Abdelrahman, D.R., Jameson, T.S., Jackman, S.R., Blackwell, J.R., Finnigan, T.J., Stephens, F.B., Dirks, M.L., and Wall, B.T. 2020. Mycoprotein ingestion stimulates protein synthesis rates to a greater extent than milk protein in rested and exercised skeletal muscle of healthy young men: a randomized controlled trial. *The American journal of clinical nutrition,* 112**,** 318-333.

99. Smiles, W.J., Parr, E.B., Coffey, V.G., Lacham-Kaplan, O., Hawley, J.A., and Camera, D.M. 2016. Protein coingestion with alcohol following strenuous exercise attenuates alcohol-induced intramyocellular apoptosis and inhibition of autophagy. *American journal of physiology. Endocrinology and metabolism,* 311**,** E836-E849.

100. Atherton, P.J., Smith, K., Etheridge, T., Rankin, D., and Rennie, M.J. 2010. Distinct anabolic signalling responses to amino acids in C2C12 skeletal muscle cells. *Amino Acids,* 38**,** 1533-9.

101. Burd, N., Holwerda, A., Selby, K., West, D., Staples, A., Cain, N., Cashaback, J., Potvin, J., Baker, S., and Phillips, S. 2010. Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *The Journal Of Physiology,* 588**,** 3119-3130.

102. Dickinson, J.M., Fry, C.S., Drummond, M.J., Gundermann, D.M., Walker, D.K., Glynn, E.L., Timmerman, K.L., Dhanani, S., Volpi, E., and Rasmussen, B.B. 2011. Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. *The Journal of nutrition,* 141**,** 856-862.

103. Layman, D.K. 2002. Role of leucine in protein metabolism during exercise and recovery. *Canadian Journal of Applied Physiology,* 27**,** 646-662.

104. Anthony, J.C., Yoshizawa, F., Anthony, T.G., Vary, T.C., Jefferson, L.S., and Kimball, S.R. 2000. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *The Journal of nutrition,* 130**,** 2413-2419.

105. Tipton, K.D., Elliott, T.A., Ferrando, A.A., Aarsland, A.A., and Wolfe, R.R. 2009. Stimulation of muscle anabolism by resistance exercise and ingestion of leucine plus protein. *Applied Physiology, Nutrition, & Metabolism = Physiologie Appliquee, Nutrition et Metabolisme,* 34**,** 151-61.

106. Wolfson, R.L., Chantranupong, L., Saxton, R.A., Shen, K., Scaria, S.M., Cantor, J.R., and Sabatini, D.M. 2016. Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science,* 351**,** 43-48.

107. Drummond, M.J. and Rasmussen, B.B. 2008. Leucine-enriched nutrients and the regulation of mTOR signalling and human skeletal muscle protein synthesis. *Current opinion in clinical nutrition and metabolic care,* 11**,** 222.

108. Waterlow, J. 1984. Protein turnover with special reference to man. *Quarterly Journal of Experimental Physiology: Translation and Integration,* 69**,** 409-438.

109. Previs, S.F., Fatica, R., Chandramouli, V., Alexander, J.C., Brunengraber, H., and Landau, B.R. 2004. Quantifying rates of protein synthesis in humans by use of 2H2O: application to patients with end-stage renal disease. *American Journal of Physiology-Endocrinology and Metabolism,* 286**,** E665-E672.

110. Jahoor, F. and Wolfe, R.R. 1987. Reassessment of primed constant-infusion tracer method to measure urea kinetics. *American Journal of Physiology-Endocrinology and Metabolism,* 252**,** E557-E564.

111. Macdonald, A.J., Small, A.C., Greig, C.A., Husi, H., Ross, J.A., Stephens, N.A., Fearon, K.C.H., and Preston, T. 2013. A novel oral tracer procedure for measurement of habitual myofibrillar protein synthesis. *Rapid Communications in Mass Spectrometry,* 27**,** 1769-1777.

112. Colley, R.C., Byrne, N.M., and Hills, A.P. 2007. Implications of the variability in time to isotopic equilibrium in the deuterium dilution technique. *European Journal of Clinical Nutrition,* 61**,** 1250-1255.

113. Watson, P.E., Watson, I.D., and Batt, R.D. 1980. Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr,* 33**,** 27-39.

114. Gasier, H.G., Fluckey, J.D., and Previs, S.F. 2010. The application of 2H2O to measure skeletal muscle protein synthesis. *Nutr Metab (Lond),* 7**,** 31.

115. Wilkinson, D.J., Franchi, M.V., Brook, M.S., Narici, M.V., Williams, J.P., Mitchell, W.K., Szewczyk, N.J., Greenhaff, P.L., Atherton, P.J., and Smith, K. 2014. A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *American Journal of Physiology-Endocrinology and Metabolism,* 306**,** E571-E579.

116. Holwerda, A.M., Paulussen, K.J., Overkamp, M., Smeets, J.S., Gijsen, A.P., Goessens, J.P., Verdijk, L.B., and van Loon, L.J. 2018. Daily resistance-type exercise stimulates muscle protein synthesis in vivo in young men. *Journal of Applied Physiology,* 124**,** 66-75.

117. Brook, M.S., Wilkinson, D.J., Mitchell, W.K., Lund, J.N., Szewczyk, N.J., Greenhaff, P.L., Smith, K., and Atherton, P.J. 2015. Skeletal muscle hypertrophy adaptations predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. *The FASEB Journal,* 29**,** 4485-4496.

118. Church, D.D., Schutzler, S.E., Wolfe, R.R., and Ferrando, A.A. 2021. Perioperative amino acid infusion reestablishes muscle net balance during total hip arthroplasty. *Physiological Reports,* 9**,** e15055.

119. Gore, D.C., Chinkes, D.L., Wolf, S.E., Sanford, A.P., Herndon, D.N., and Wolfe, R.R. 2006. Quantification of Protein Metabolism *in Vivo* for Skin, Wound, and Muscle in Severe Burn Patients. *Journal of Parenteral and Enteral Nutrition,* 30**,** 331-338.

120. Sheffield-Moore, M., Dillon, E., Randolph, K., Casperson, S., White, G., Jennings, K., Rathmacher, J., Schuette, S., Janghorbani, M., and Urban, R. 2014. Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss. *Journal of cachexia, sarcopenia and muscle,* 5**,** 19-25.

121. Yarasheski, K.E., Zachwieja, J.J., and Bier, D.M. 1993. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *American Journal of Physiology-Endocrinology and Metabolism,* 265**,** E210-E214.

122. Lecker, S.H., Solomon, V., Mitch, W.E., and Goldberg, A.L. 1999. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. *The Journal of nutrition,* 129**,** 227S-237S.

123. Bodine, S.C., Latres, E., Baumhueter, S., Lai, V.K.-M., Nunez, L., Clarke, B.A., Poueymirou, W.T., Panaro, F.J., Na, E., and Dharmarajan, K. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science,* 294**,** 1704-1708.

124. Dickinson, J.M., Reidy, P.T., Gundermann, D.M., Borack, M.S., Walker, D.K., D'Lugos, A.C., Volpi, E., and Rasmussen, B.B. 2017. The impact of postexercise essential amino acid ingestion on the ubiquitin proteasome and autophagosomal-lysosomal systems in skeletal muscle of older men. *J Appl Physiol (1985),* 122**,** 620-630.

125. Belcastro, A.N. 1993. Skeletal muscle calcium-activated neutral protease (calpain) with exercise. *Journal of Applied Physiology,* 74**,** 1381-1386.

126. Du, J., Wang, X., Miereles, C., Bailey, J.L., Debigare, R., Zheng, B., Price, S.R., and Mitch, W.E. 2004. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *Journal of Clinical Investigation,* 113**,** 115-123.

127. Draganidis, D., Chondrogianni, N., Chatzinikolaou, A., Terzis, G., Karagounis, L.G., Sovatzidis, A., Avloniti, A., Lefaki, M., Protopapa, M., Deli, C.K., Papanikolaou, K., Jamurtas, A.Z., and Fatouros, I.G. 2017. Protein ingestion preserves proteasome activity during intense aseptic inflammation and facilitates skeletal muscle recovery in humans. *British Journal of Nutrition,* 118**,** 189-200.

128. Gregorio, C.C., Perry, C.N., and McElhinny, A.S. 2005. Functional properties of the titin/connectin-associated proteins, the muscle-specific RING finger proteins (MURFs), in striated muscle. *J Muscle Res Cell Motil,* 26**,** 389-400.

129. Fry, C.S., Drummond, M.J., Glynn, E.L., Dickinson, J.M., Gundermann, D.M., Timmerman, K.L., Walker, D.K., Volpi, E., and Rasmussen, B.B. 2013. Skeletal muscle autophagy and protein breakdown following resistance exercise are similar in younger and older adults. *J Gerontol A Biol Sci Med Sci,* 68**,** 599-607.

130. Glynn, E.L., Fry, C.S., Drummond, M.J., Dreyer, H.C., Dhanani, S., Volpi, E., and Rasmussen, B.B. 2010. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 299**,** R533-R540.

131. Urso, M.L., Scrimgeour, A.G., Chen, Y.-W., Thompson, P.D., and Clarkson, P.M. 2006. Analysis of human skeletal muscle after 48 h immobilization reveals alterations in mRNA and protein for extracellular matrix components. *Journal of Applied Physiology,* 101**,** 1136-1148.

132. Wall, B.T., Dirks, M.L., Snijders, T., Senden, J.M., Dolmans, J., and Van Loon, L.J. 2014. Substantial skeletal muscle loss occurs during only 5 days of disuse. *Acta physiologica,* 210**,** 600-611.

133. Jameson, T.S.O., Kilroe, S.P., Fulford, J., Abdelrahman, D.R., Murton, A.J., Dirks, M.L., Stephens, F.B., and Wall, B.T. 2021. Muscle damaging eccentric exercise attenuates disuse-induced declines in daily myofibrillar protein synthesis and transiently prevents muscle atrophy in healthy men. *American Journal of Physiology - Endocrinology & Metabolism,* 321**,** E674-E688.

134. Greenhaff, P.L., Karagounis, L., Peirce, N., Simpson, E.J., Hazell, M., Layfield, R., Wackerhage, H., Smith, K., Atherton, P., and Selby, A. 2008. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *American Journal of Physiology-Endocrinology and Metabolism,* 295**,** E595-E604.

135. Børsheim, E., Cree, M.G., Tipton, K.D., Elliott, T.A., Aarsland, A., and Wolfe, R.R. 2004. Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *Journal of applied physiology,* 96**,** 674-678.

136. Wolfe, R.R. and Chinkes, D.L., *Isotope tracers in metabolic research: principles and practice of kinetic analysis*. 2004: John Wiley & Sons.

137. Biolo, G., Fleming, R., Maggi, S.P., and Wolfe, R.R. 1995. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *American Journal of Physiology-Endocrinology And Metabolism,* 268**,** E75-E84.

138. Zhang, X., Chinkes, D.L., Sakurai, Y., and Wolfe, R.R. 1996. An isotopic method for measurement of muscle protein fractional breakdown rate in vivo. *American Journal of Physiology-Endocrinology And Metabolism,* 270**,** E759-E767.

139. Pavis, G.F., Abdelrahman, D.R., Murton, A.J., Wall, B.T., Stephens, F.B., and Dirks, M.L. 2023. Short-term disuse does not affect postabsorptive or postprandial muscle protein fractional breakdown rates. *Journal of Cachexia, Sarcopenia and Muscle*.

140. Tischler, M.E. and Fagan, J.M. 1983. Response to trauma of protein, amino acid, and carbohydrate metabolism in injured and uninjured rat skeletal muscles. *Metabolism,* 32**,** 853-868.

141. Koopman, R., Wagenmakers, A.J., Manders, R.J., Zorenc, A.H., Senden, J.M., Gorselink, M., Keizer, H.A., and van Loon, L.J. 2005. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *American Journal of Physiology-Endocrinology and Metabolism,* 288**,** E645-E653.

142. Kumar, V., Selby, A., Rankin, D., Patel, R., Atherton, P., Hildebrandt, W., Williams, J., Smith, K., Seynnes, O., Hiscock, N., and Rennie, M.J. 2009. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of Physiology,* 587**,** 211-217.

143. Burd, N.A., Andrews, R.J., West, D.W.D., Little, J.P., Cochran, A.J.R., Hector, A.J., Cashaback, J.G.A., Gibala, M.J., Potvin, J.R., Baker, S.K., and Phillips, S.M. 2012. Muscle time under tension during resistance exercise stimulates differential muscle protein sub-fractional synthetic responses in men. *The Journal of Physiology,* 590**,** 351-62.

144. Moore, D.R., Phillips, S.M., Babraj, J.A., Smith, K., and Rennie, M.J. 2005. Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *Am J Physiol Endocrinol Metab,* 288**,** E1153-9.

145. McKendry, J., Perez-Lopez, A., McLeod, M., Luo, D., Dent, J.R., Smeuninx, B., Yu, J., Taylor, A.E., Philp, A., and Breen, L. 2016. Short inter-set rest blunts resistance exercise-induced increases in myofibrillar protein synthesis and intracellular signalling in young males. *Experimental Physiology,* 101**,** 866-82.

146. Burd, N.A., Mitchell, C.J., Churchward-Venne, T.A., and Phillips, S.M. 2012. Bigger weights may not beget bigger muscles: evidence from acute muscle protein synthetic responses after resistance exercise. *Applied Physiology, Nutrition, and Metabolism,* 37**,** 551-554.

147. MacDougall, J.D., Gibala, M.J., Tarnopolsky, M.A., MacDonald, J.R., Interisano, S.A., and Yarasheski, K.E. 1995. The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can J Appl Physiol,* 20**,** 480-6.

148. Buckner, S.L., Mouser, J.G., Jessee, M.B., Dankel, S.J., Mattocks, K.T., and Loenneke, J.P. 2017. What does individual strength say about resistance training status? *Muscle & nerve,* 55**,** 455-457.

149. Maughan, R., Watson, J., and Weir, J. 1984. Muscle strength and cross-sectional area in man: a comparison of strength-trained and untrained subjects. *British journal of sports medicine,* 18**,** 149-157.

150. Phillips, S.M., Tipton, K., Ferrando, A.A., and Wolfe, R.R. 1999. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *American Journal of Physiology-Endocrinology and Metabolism*.

151. Kim, P.L., Staron, R.S., and Phillips, S.M. 2005. Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *The Journal of Physiology,* 568**,** 283-290.

152. Wilkinson, S.B., Phillips, S.M., Atherton, P.J., Patel, R., Yarasheski, K.E., Tarnopolsky, M.A., and Rennie, M.J. 2008. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *The Journal of Physiology,* 586**,** 3701-3717.

153. Tang, J.E., Perco, J.G., Moore, D.R., Wilkinson, S.B., and Phillips, S.M. 2008. Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol,* 294**,** R172-8.

154. Damas, F., Phillips, S.M., Lixandrão, M.E., Vechin, F.C., Libardi, C.A., Roschel, H., Tricoli, V., and Ugrinowitsch, C. 2016. Early resistance training-induced increases in muscle cross-sectional area are concomitant with edema-induced muscle swelling. *European Journal of Applied Physiology,* 116**,** 49-56.

155. Wolfe, R.R. 2002. Regulation of muscle protein by amino acids. *The Journal of nutrition,* 132**,** 3219S-3224S.

156. Groen, B.B.L., Horstman, A.M., Hamer, H.M., De Haan, M., Van Kranenburg, J., Bierau, J., Poeze, M., Wodzig, W.K.W.H., Rasmussen, B.B., and Van Loon, L.J.C. 2015. Post-Prandial Protein Handling: You Are What You Just Ate. *PLOS ONE,* 10**,** e0141582.

157. Bohé, J., Low, J.A., Wolfe, R.R., and Rennie, M.J. 2001. Rapid report: Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *The Journal of physiology,* 532**,** 575-579.

158. Atherton, P.J., Etheridge, T., Watt, P.W., Wilkinson, D., Selby, A., Rankin, D., Smith, K., and Rennie, M.J. 2010. Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *The American journal of clinical nutrition,* 92**,** 1080-1088.

159. Breen, L. and Phillips, S.M. 2011. Skeletal muscle protein metabolism in the elderly: Interventions to counteract the'anabolic resistance'of ageing. *Nutrition & metabolism,* 8**,** 1-11.

160. Tang, J.E., Moore, D.R., Kujbida, G.W., Tarnopolsky, M.A., and Phillips, S.M. 2009. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol (1985),* 107**,** 987-92.

161. Moore, D.R., Robinson, M.J., Fry, J.L., Tang, J.E., Glover, E.I., Wilkinson, S.B., Prior, T., Tarnopolsky, M.A., and Phillips, S.M. 2009. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *The American journal of clinical nutrition,* 89**,** 161-168.

162. Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P.J., Wackerhage, H., Taylor, P.M., and Rennie, M.J. 2005. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *Am J Physiol Endocrinol Metab,* 19**,** 1-22.

163. Apro, W., Moberg, M., Hamilton, D.L., Ekblom, B., Rooyackers, O., Holmberg, H.C., and Blomstrand, E. 2015. Leucine does not affect mechanistic target of rapamycin complex 1 assembly but is required for maximal ribosomal protein s6 kinase 1 activity in human skeletal muscle following resistance exercise. *FASEB J,* 29**,** 4358-73.

164. Churchward-Venne, T.A., Burd, N., Mitchell, C., West, D., Philp, A., Marcotte, G., Baker, S., Baar, K., and Phillips, S. 2012. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *The Journal Of Physiology,* 590**,** 2751-2765.

165. Katsanos, C.S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., and Wolfe, R.R. 2006. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *American Journal of Physiology-Endocrinology and Metabolism,* 291**,** E381-E387.

166. Wall, B.T., Hamer, H.M., de Lange, A., Kiskini, A., Groen, B.B., Senden, J.M.G., Gijsen, A.P., Verdijk, L.B., and van Loon, L.J. 2013. Leucine co-ingestion improves post-prandial muscle protein accretion in elderly men. *Clinical nutrition,* 32**,** 412-419.

167. Rieu, I., Balage, M., Sornet, C., Giraudet, C., Pujos, E., Grizard, J., Mosoni, L., and Dardevet, D. 2006. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *The Journal of Physiology,* 575**,** 305-315.

168. Witard, O.C., Jackman, S.R., Breen, L., Smith, K., Selby, A., and Tipton, K.D. 2014. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *American Journal of Clinical Nutrition,* 99**,** 86-95.

169. Moore, D.R., Robinson, M.J., Fry, J.L., Tang, J.E., Glover, E.I., Wilkinson, S.B., Prior, T., Tarnopolsky, M.A., and Phillips, S.M. 2009. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr,* 89**,** 161-8.

170. Moore, D.R., Tang, J.E., Burd, N.A., Rerecich, T., Tarnopolsky, M.A., and Phillips, S.M. 2009. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol,* 587**,** 897-904.

171. Dreyer, H., Drummond, M., Pennings, B., Fujita, S., Glynn, E., Chinkes, D., Dhanani, S., Volpi, E., and Rasmussen, B. 2008. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *Am J Physiol Endocrinol Metab,* 294**,** E392-400.

172. Cermak, N.M., Res, P.T., de Groot, L.C., Saris, W.H., and van Loon, L.J. 2012. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *The American journal of clinical nutrition,* 96**,** 1454-1464.

173. Wilkinson, S.B., Tarnopolsky, M.A., MacDonald, M.J., MacDonald, J.R., Armstrong, D., and Phillips, S.M. 2007. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. *The American journal of clinical nutrition,* 85**,** 1031-1040.

174. Pennings, B., Boirie, Y., Senden, J.M., Gijsen, A.P., Kuipers, H., and van Loon, L.J. 2011. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *The American journal of clinical nutrition,* 93**,** 997-1005.

175. Burd, N., Yang, Y., Moore, D., Tang, J., Tarnopolsky, M., and Phillips, S. 2012. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. *British Journal of Nutrition,* 108**,** 958-962.

176. Van Vliet, S., Shy, E.L., Abou Sawan, S., Beals, J.W., West, D.W., Skinner, S.K., Ulanov, A.V., Li, Z., Paluska, S.A., and Parsons, C.M. 2017. Consumption of whole eggs promotes greater stimulation of postexercise muscle protein synthesis than consumption of isonitrogenous amounts of egg whites in young men. *The American journal of clinical nutrition,* 106**,** 1401-1412.

177. Fuchs, C.J., Hermans, W.J., Smeets, J.S., Senden, J.M., van Kranenburg, J., Gorissen, S.H., Burd, N.A., Verdijk, L.B., and van Loon, L.J. 2022. Raw eggs to support post-exercise recovery in healthy young men: did Rocky get it right or wrong? *The Journal of Nutrition*.

178. Burd, N.A., Gorissen, S.H., Van Vliet, S., Snijders, T., and Van Loon, L.J. 2015. Differences in postprandial protein handling after beef compared with milk ingestion during postexercise recovery: a randomized controlled trial. *The American journal of clinical nutrition,* 102**,** 828-836.

179. Churchward-Venne, T.A., Burd, N.A., and Phillips, S.M. 2012. Nutritional regulation of muscle protein synthesis with resistance exercise: strategies to enhance anabolism. *Nutrition & Metabolism,* 9**,** 1-8.

180. Pinckaers, P.J.M., Trommelen, J., Snijders, T., and Van Loon, L.J.C. 2021. The Anabolic Response to Plant-Based Protein Ingestion. *Sports Medicine,* 51**,** 59-74.

181. Tipton, K.D., Elliott, T.A., Cree, M.G., Wolf, S.E., Sanford, A.P., and Wolfe, R.R. 2004. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. *Medicine & Science in Sports & Exercise,* 36**,** 2073-2081.

182. Borack, M.S., Reidy, P.T., Husaini, S.H., Markofski, M.M., Deer, R.R., Richison, A.B., Lambert, B.S., Cope, M.B., Mukherjea, R., Jennings, K., Volpi, E., and Rasmussen, B.B. 2016. Soy-Dairy Protein Blend or Whey Protein Isolate Ingestion Induces Similar Postexercise Muscle Mechanistic Target of Rapamycin Complex 1 Signaling and Protein Synthesis Responses in Older Men. *J Nutr,* 146**,** 2468-2475.

183. Burd, N.A., West, D.W., Moore, D.R., Atherton, P.J., Staples, A.W., Prior, T., Tang, J.E., Rennie, M.J., Baker, S.K., and Phillips, S.M. 2011. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J Nutr,* 141**,** 568-73.

184. Pasiakos, S.M., Lieberman, H.R., and McLellan, T.M. 2014. Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: a systematic review. *Sports Med,* 44**,** 655-70.

185. Cockburn, E., Robson-Ansley, P., Hayes, P.R., and Stevenson, E. 2012. Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage. *European Journal of Applied Physiology,* 112**,** 3187-3194.

186. Jameson, T.S.O., Pavis, G.F., Dirks, M.L., Lee, B.P., Abdelrahman, D.R., Murton, A.J., Porter, C., Alamdari, N., Mikus, C.R., Wall, B.T., and Stephens, F.B. 2021. Reducing NF-kappaB Signaling Nutritionally is Associated with Expedited Recovery of Skeletal

Muscle Function After Damage. *Journal of Clinical Endocrinology & Metabolism,* 106**,** 2057-2076.

187. Davies, R.W., Carson, B.P., and Jakeman, P.M. 2018. The effect of whey protein supplementation on the temporal recovery of muscle function following resistance training: A systematic review and meta-analysis. *Nutrients,* 10**,** 221.

188. Cooke, M.B., Rybalka, E., Stathis, C.G., Cribb, P.J., and Hayes, A. 2010. Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. *J Int Soc Sports Nutr,* 7**,** 30.

189. Lauritzen, F., Paulsen, G., Raastad, T., Bergersen, L.H., and Owe, S.G. 2009. Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. *Journal of Applied Physiology,* 107**,** 1923-1934.

190. Nosaka, K., Clarkson, P.M., McGuiggin, M.E., and Byrne, J.M. 1991. Time course of muscle adaptation after high force eccentric exercise. *European journal of applied physiology and occupational physiology,* 63**,** 70-76.

191. Crameri, R.M., Langberg, H., Magnusson, P., Jensen, C.H., Schrøder, H.D., Olesen, J.L., Suetta, C., Teisner, B., and Kjaer, M. 2004. Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *The Journal of Physiology,* 558**,** 333-340.

192. Mackey, A.L., Rasmussen, L.K., Kadi, F., Schjerling, P., Helmark, I.C., Ponsot, E., Aagaard, P., Durigan, J.L.Q., and Kjaer, M. 2016. Activation of satellite cells and the regeneration of human skeletal muscle are expedited by ingestion of nonsteroidal anti-inflammatory medication. *The FASEB Journal,* 30**,** 2266-2281.

193. Farup, J., Rahbek, S.K., Knudsen, I.S., de Paoli, F., Mackey, A.L., and Vissing, K. 2014. Whey protein supplementation accelerates satellite cell proliferation during recovery from eccentric exercise. *Amino Acids,* 46**,** 2503-2516.

194. Rahbek, S.K., Farup, J., Møller, A.B., Vendelbo, M.H., Holm, L., Jessen, N., and Vissing, K. 2014. Effects of divergent resistance exercise contraction mode and dietary supplementation type on anabolic signalling, muscle protein synthesis and muscle hypertrophy. *Amino Acids,* 46**,** 2377-2392.

195. Waskiw-Ford, M., Hannaian, S., Duncan, J., Kato, H., Abou Sawan, S., Locke, M., Kumbhare, D., and Moore, D. 2020. Leucine-Enriched Essential Amino Acids Improve Recovery from Post-Exercise Muscle Damage Independent of Increases in Integrated Myofibrillar Protein Synthesis in Young Men. *Nutrients,* 12**,** 1061.

196. Van Loon, L.J.C., Boirie, Y., Gijsen, A.P., Fauquant, J., de Roos, A.L., Kies, A.K., Lemosquet, S., Saris, W.H., and Koopman, R. 2009. The production of intrinsically labeled milk protein provides a functional tool for human nutrition research. *Journal of Dairy Science,* 92**,** 4812-22.

197. Yang, Y., Churchward-Venne, T.A., Burd, N.A., Breen, L., Tarnopolsky, M.A., and Phillips, S.M. 2012. Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. *Nutrition and Metabolism,* 9.

198. Pennings, B., Groen, B., de Lange, A., Gijsen, A.P., Zorenc, A.H., Senden, J.M.G., and van Loon, L.J.C. 2012. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *American Journal of Physiology - endocrinology and metabolism,* 302**,** E992-E999.

199. West, D.W., Burd, N.A., Coffey, V.G., Baker, S.K., Burke, L.M., Hawley, J.A., Moore, D.R., Stellingwerff, T., and Phillips, S.M. 2011. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise–. *The American journal of clinical nutrition,* 94**,** 795-803.

200. Tipton, K.D., Gurkin, B.E., Matin, S., and Wolfe, R.R. 1999. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *The Journal of Nutritional Biochemistry,* 10**,** 89-95.

201. Kim, I.-Y., Schutzler, S., Schrader, A., Spencer, H.J., Azhar, G., Ferrando, A.A., and Wolfe, R.R. 2016. The anabolic response to a meal containing different amounts of protein is not limited by the maximal stimulation of protein synthesis in healthy young adults. *American journal of physiology-endocrinology and metabolism,* 310**,** E73-E80.

202. West, S., Monteyne, A.J., Whelehan, G., Abdelrahman, D.R., Murton, A.J., Finnigan, T.J., Blackwell, J.R., Stephens, F.B., and Wall, B.T. 2022. Mycoprotein ingestion within or without its wholefood matrix results in equivalent stimulation of myofibrillar protein synthesis rates in resting and exercised muscle of young men. *British Journal of Nutrition***,** 1-13.

203. West, D.W.D., Burd, N.A., Coffey, V.G., Baker, S.K., Burke, L.M., Hawley, J.A., Moore, D.R., Stellingwerff, T., and Phillips, S.M. 2011. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *The American journal of clinical nutrition,* 94**,** 795-803.

204. Areta, J.L., Burke, L.M., Ross, M.L., Camera, D.M., West, D.W., Broad, E.M., Jeacocke, N.A., Moore, D.R., Stellingwerff, T., and Phillips, S.M. 2013. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of physiology,* 591**,** 2319-2331.

205. Volpi, E., Kobayashi, H., Sheffield-Moore, M., Mittendorfer, B., and Wolfe, R.R. 2003. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *The American journal of clinical nutrition,* 78**,** 250-258.

206. World Health Organization, F.U.N.U., *Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation*. Technical Report Series no. 935 ed. 2007, Geneva: World Health Organization.

207. Gorissen, S.H., Crombag, J.J., Senden, J.M., Waterval, W.H., Bierau, J., Verdijk, L.B., and van Loon, L.J. 2018. Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino acids,* 50**,** 1685-1695.

208. van Vliet, S., Burd, N.A., and van Loon, L.J. 2015. The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. *J Nutr,* 145**,** 1981-91.

209. Devi, S., Varkey, A., Sheshshayee, M.S., Preston, T., and Kurpad, A.V. 2018. Measurement of protein digestibility in humans by a dual-tracer method. *The American Journal of Clinical Nutrition,* 107**,** 984-991.

210. Pennings, B., Koopman, R., Beelen, M., Senden, J.M., Saris, W.H., and Van Loon, L.J. 2011. Exercising before protein intake allows for greater use of dietary protein–derived amino acids for de novo muscle protein synthesis in both young and elderly men. *The American Journal of Clinical Nutrition,* 93**,** 322-331.

211. Burd, N.A., Beals, J.W., Martinez, I.G., Salvador, A.F., and Skinner, S.K. 2019. Food-first approach to enhance the regulation of post-exercise skeletal muscle protein synthesis and remodeling. *Sports medicine,* 49**,** 59-68.

212. Koopman, R., Crombach, N., Gijsen, A.P., Walrand, S., Fauquant, J., Kies, A.K., Lemosquet, S., Saris, W.H., Boirie, Y., and van Loon, L.J. 2009. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *The American journal of clinical nutrition,* 90**,** 106-115.

213. Burd, N.A., Yang, Y., Moore, D.R., Tang, J.E., Tarnopolsky, M.A., and Phillips, S.M. 2012. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. *British Journal of Nutrition,* 108**,** 958-962.

214. Churchward-Venne, T.A., Breen, L., Di Donato, D.M., Hector, A.J., Mitchell, C.J., Moore, D.R., Stellingwerff, T., Breuille, D., Offord, E.A., and Baker, S.K. 2014. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. *The American Journal of Clinical Nutrition,* 99**,** 276-286.

215. Chan, A., D'Souza, R., Beals, J., Zeng, N., Prodhan, U., Fanning, A., Poppitt, S., Li, Z., Burd, N., Cameron-Smith, D., and et al. 2019. The Degree of Aminoacidemia after Dairy Protein Ingestion Does Not Modulate the Postexercise Anabolic Response in Young Men: a Randomized Controlled Trial. 149**,** 1511-1522.

216. Mitchell, C.J., McGregor, R.A., D'Souza, R.F., Thorstensen, E.B., Markworth, J.F., Fanning, A.C., Poppitt, S.D., and Cameron-Smith, D. 2015. Consumption of milk protein or whey protein results in a similar increase in muscle protein synthesis in middle aged men. *Nutrients,* 7**,** 8685-8699.

217. Zaromskyte, G., Prokopidis, K., Ioannidis, T., Tipton, K.D., and Witard, O.C. 2021. Evaluating the leucine trigger hypothesis to explain the postprandial regulation of muscle protein synthesis in young and older adults: A systematic review. *Frontiers in Nutrition,* 8**,** 402.

218. Hermans, W.J.H., Senden, J.M., Churchward-Venne, T.A., Paulussen, K.J.M., Fuchs, C.J., Smeets, J.S.J., van Loon, J.J.A., Verdijk, L.B., and van Loon, L.J.C. 2021. Insects are a viable protein source for human consumption: from insect protein digestion to postprandial muscle protein synthesis in vivo in humans: a double-blind randomized trial. *Am J Clin Nutr,* 114**,** 934-944.

219. Lea, E.J., Crawford, D., and Worsley, A. 2006. Consumers' readiness to eat a plant-based diet. *European Journal of Clinical Nutrition,* 60**,** 342-351.

220. Henchion, M., Hayes, M., Mullen, A., Fenelon, M., and Tiwari, B. 2017. Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods,* 6**,** 53.

221. Donati, M., Menozzi, D., Zighetti, C., Rosi, A., Zinetti, A., and Scazzina, F. 2016. Towards a sustainable diet combining economic, environmental and nutritional objectives. *Appetite,* 106**,** 48-57.

222. Sabaté, J. and Wien, M. 2015. A perspective on vegetarian dietary patterns and risk of metabolic syndrome. *British Journal of Nutrition,* 113**,** S136-S143.

223. Tuso, P.J., Ismail, M.H., Ha, B.P., and Bartolotto, C. 2013. Nutritional Update for Physicians: Plant-Based Diets. *The Permanente Journal,* 17**,** 61-66.

224. Churchward-Venne, T.A., Pinckaers, P.J., Smeets, J.S., Peeters, W.M., Zorenc, A.H., Schierbeek, H., Rollo, I., Verdijk, L.B., and van Loon, L.J. 2019. Myofibrillar and mitochondrial protein synthesis rates do not differ in young men following the ingestion of carbohydrate with whey, soy, or leucine-enriched soy protein after

concurrent resistance-and endurance-type exercise. *The Journal of nutrition,* 149**,** 210-220.

225. Gorissen, S.H., Horstman, A.M., Franssen, R., Crombag, J.J., Langer, H., Bierau, J., Respondek, F., and van Loon, L.J. 2016. Ingestion of Wheat Protein Increases In Vivo Muscle Protein Synthesis Rates in Healthy Older Men in a Randomized Trial. *J Nutr,* 146**,** 1651-9.

226. Reidy, P.T., Walker, D.K., Dickinson, J.M., Gundermann, D.M., Drummond, M.J., Timmerman, K.L., Cope, M.B., Mukherjea, R., Jennings, K., and Volpi, E. 2014. Soy-dairy protein blend and whey protein ingestion after resistance exercise increases amino acid transport and transporter expression in human skeletal muscle. *Journal of Applied Physiology,* 116**,** 1353-1364.

227. Borack, M., Reidy, P., Husaini, S., Markofski, M., Deer, R., Richison, A., Lambert, B., Cope, M., Mukherjea, R., Jennings, K., and et al. 2016. Effect of soy-dairy protein blend ingestion on post-exercise muscle mtorc1 signaling and protein synthesis in older adults. *The Journal of Nutrition,* 30.

228. Pinckaers, P.J., Kouw, I.W., Gorissen, S.H., Houben, L.H., Senden, J.M., Wodzig, W.K., de Groot, L.C., Verdijk, L.B., Snijders, T., and van Loon, L.J. 2022. The Muscle Protein Synthetic Response to the Ingestion of a Plant-Derived Protein Blend Does Not Differ from an Equivalent Amount of Milk Protein in Healthy Young Males. *The Journal of Nutrition,* 152**,** 2734-2743.

229. Pinckaers, P.J.M., Kouw, I.W.K., Hendriks, F.K., Van Kranenburg, J.M.X., De Groot, L.C.P.G.M., Verdijk, L.B., Snijders, T., and Van Loon, L.J.C. 2021. No differences in muscle protein synthesis rates following ingestion of wheat protein, milk protein, and their protein blend in healthy, young males. *British Journal of Nutrition,* 126**,** 1832-1842.

230. Pinckaers, P.J.M., Hendriks, F.K., Hermans, W.J.H., Goessens, J.P.B., Senden, J.M., van Kranenburg, J.M.X., Wodzig, W., Snijders, T., and van Loon, L.J.C. 2022. Potato Protein Ingestion Increases Muscle Protein Synthesis Rates at Rest and during Recovery from Exercise in Humans. *Medicine and science in sports and exercise*.

231. Reidy, P., Walker, D.K., Dickinson, J.M., Gundermann, D.M., Drummond, M.J., Timmerman, K.L., Fry, C.S., Borack, M.S., Cope, M.B., and Mukherjea, R. 2013. Protein blend ingestion following resistance exercise promotes human muscle protein synthesis. *The Journal of nutrition,* 143**,** 410-416.

232. Kouw, I.W.K., Pinckaers, P.J.M., Le Bourgot, C., Van Kranenburg, J.M.X., Zorenc, A.H., De Groot, L.C.P.G.M., Verdijk, L., Snijders, T., and Van Loon, L.J.C. 2022. Ingestion of an ample amount of meat substitute based on a lysine-enriched, plant-based protein blend stimulates postprandial muscle protein synthesis to a similar extent as an isonitrogenous amount of chicken in healthy, young men. *British Journal of Nutrition,* 128**,** 1955-1965.

233. Norton, L.E., Wilson, G.J., Layman, D.K., Moulton, C.J., and Garlick, P.J. 2012. Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. *Nutrition & metabolism,* 9**,** 67.

234. Shanthakumar, P., Klepacka, J., Bains, A., Chawla, P., Dhull, S.B., and Najda, A. 2022. The Current Situation of Pea Protein and Its Application in the Food Industry. *Molecules,* 27**,** 5354.

235. Dahl, W.J., Foster, L.M., and Tyler, R.T. 2012. Review of the health benefits of peas (*Pisum sativum*.). *British Journal of Nutrition,* 108**,** S3-S10.

236. Boye, J., Zare, F., and Pletch, A. 2010. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food research international,* 43**,** 414-431.

237. Ge, J., Sun, C.X., Corke, H., Gul, K., Gan, R.Y., and Fang, Y. 2020. The health benefits, functional properties, modifications, and applications of pea (*Pisum sativum*) protein: Current status, challenges, and perspectives. *Comprehensive Reviews in Food Science and Food Safety,* 19**,** 1835-1876.

238. Robinson, G.H.J. and Domoney, C. 2021. Perspectives on the genetic improvement of health- and nutrition-related traits in pea. *Plant Physiol Biochem,* 158**,** 353-362.

239. Kaden, S. 2020. Health Benefits of Pea Protein Isolate: A Comparative Review. *SURG Journal,* 12.

240. Mariotti, F., Pueyo, M.E., Tome, D., Berot, S., Benamouzig, R., and Mahe, S. 2001. The influence of the albumin fraction on the bioavailability and postprandial utilization of pea protein given selectively to humans. *The Journal of nutrition,* 131**,** 1706-1713.

241. Kumar, M., Tomar, M., Potkule, J., Verma, R., Punia, S., Mahapatra, A., Belwal, T., Dahuja, A., Joshi, S., Berwal, M.K., Satankar, V., Bhoite, A.G., Amarowicz, R., Kaur, C., and Kennedy, J.F. 2021. Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids,* 115.

242. Babault, N., Païzis, C., Deley, G., Guérin-Deremaux, L., Saniez, M.-H., Lefranc-Millot, C., and Allaert, F.A. 2015. Pea proteins oral supplementation promotes muscle thickness gains during resistance training: a double-blind, randomized, Placebo-controlled clinical trial vs. Whey protein. *Journal of the International Society of Sports Nutrition,* 12**,** 1-9.

243. Nieman, D.C., Zwetsloot, K.A., Simonson, A.J., Hoyle, A.T., Wang, X., Nelson, H.K., Lefranc-Millot, C., and Guérin-Deremaux, L. 2020. Effects of Whey and Pea Protein Supplementation on Post-Eccentric Exercise Muscle Damage: A Randomized Trial. *Nutrients,* 12**,** 2382.

244. Lanng, S.K., Oxfeldt, M., Pedersen, S.S., Johansen, F.T., Risikesan, J., Lejel, T., Bertram, H.C., and Hansen, M. 2023. Influence of protein source (cricket, pea, whey) on amino acid bioavailability and activation of the mTORC1 signaling pathway after resistance exercise in healthy young males. *European Journal of Nutrition,* 62**,** 1295-1308.

245. West, S., Monteyne, A.J., Whelehan, G., van der Heijden, I., Abdelrahman, D.R., Murton, A.J., Finnigan, T.J., Stephens, F.B., and Wall, B.T. 2023. Ingestion of mycoprotein, pea protein and their blend support comparable post-exercise myofibrillar protein synthesis rates in resistance trained individuals. *American Journal of Physiology-Endocrinology and Metabolism*.

246. Young, V.R. and Pellett, P.L. 1994. Plant proteins in relation to human protein and amino acid nutrition. *The American journal of clinical nutrition,* 59**,** 1203S-1212S.

247. Herrlinger, K.A., Chirouzes, D.M., and Ceddia, M.A. 2015. Supplementation with a polyphenolic blend improves post-exercise strength recovery and muscle soreness. *Food & nutrition research,* 59**,** 30034.

248. MacInnis, M.J., McGlory, C., Gibala, M.J., and Phillips, S.M. 2017. Investigating human skeletal muscle physiology with unilateral exercise models: when one limb is more powerful than two. *Applied Physiology, Nutrition, and Metabolism,* 42**,** 563-570.

249. Cheng, A.J. and Rice, C.L. 2005. Fatigue and recovery of power and isometric torque following isotonic knee extensions. *Journal of Applied Physiology,* 99**,** 1446-1452.

250. Jones, D., Newham, D., and Torgan, C. 1989. Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. *The Journal of physiology,* 412**,** 415-427.

251. Bijur, P.E., Silver, W., and Gallagher, E.J. 2001. Reliability of the Visual Analog Scale for Measurement of Acute Pain. *Academic Emergency Medicine,* 8**,** 1153-1157.

252. Jameson, T.S.O., Islam, H., Wall, B.T., Little, J.P., and Stephens, F.B. 2022. Oral ketone monoester supplementation does not accelerate recovery of muscle force or modulate circulating cytokine concentrations after muscle-damaging eccentric exercise in healthy males and females. *Experimental Physiology,* 107**,** 1339-1348.

253. Henry, C. 2005. Basal metabolic rate studies in humans: measurement and development of new equations. *Public health nutrition,* 8**,** 1133-1152.

254. Phillips, S.M. 2004. Protein requirements and supplementation in strength sports. *Nutrition,* 20**,** 689-695.

255. Morton, R.W., Murphy, K.T., McKellar, S.R., Schoenfeld, B.J., Henselmans, M., Helms, E., Aragon, A.A., Devries, M.C., Banfield, L., Krieger, J.W., and Phillips, S.M. 2018. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *British Journal of Sports Medicine,* 52**,** 376-384.

256. Watson, P.E., Watson, I.D., and Batt, R.D. 1980. Total body water volumes for adult males and females estimated from simple anthropometric measurements. *The American journal of clinical nutrition,* 33**,** 27-39.

257. Tarnopolsky, M.A., Pearce, E., Smith, K., and Lach, B. 2011. Suction-modified Bergström muscle biopsy technique: Experience with 13,500 procedures. *Muscle & Nerve,* 43**,** 716-725.

258. Molnár-Perl, I. and Katona, Z.F. 2000. GC-MS of amino acids as their trimethylsilyl/t-butyldimethylsilyl Derivatives: In model solutions III. *Chromatographia,* 51**,** S228-S236.

259. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. 2012. Fiji: an open-source platform for biological-image analysis. *Nature Methods,* 9**,** 676-682.

260. Farup, J., Kjølhede, T., Sørensen, H., Dalgas, U., Møller, A.B., Vestergaard, P.F., Ringgaard, S., Bojsen-Møller, J., and Vissing, K. 2012. Muscle Morphological and Strength Adaptations to Endurance Vs. Resistance Training. *Journal of Strength and Conditioning Research,* 26**,** 398-407.

261. Van Vliet, S., Beals, J.W., Holwerda, A.M., Emmons, R.S., Goessens, J.P., Paluska, S.A., De Lisio, M., Van Loon, L.J., and Burd, N.A. 2019. Time-dependent regulation of postprandial muscle protein synthesis rates after milk protein ingestion in young men. *Journal of Applied Physiology,* 127**,** 1792-1801.

262. Moore, D.R., Tang, J.E., Burd, N.A., Rerecich, T., Tarnopolsky, M.A., and Phillips, S.M. 2009. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *The Journal of physiology,* 587**,** 897-904.

263. Burd, N.A., West, D.W., Moore, D.R., Atherton, P.J., Staples, A.W., Prior, T., Tang, J.E., Rennie, M.J., Baker, S.K., and Phillips, S.M. 2011. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *The Journal of Nutrition,* 141**,** 568-573.

264. Tipton, K.D., Elliott, T.A., Cree, M.G., Aarsland, A.A., Sanford, A.P., and Wolfe, R.R. 2007. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *American Journal of Physiology - Endocrinology & Metabolism,* 292**,** E71-6.

265. Burke, L.M., Hawley, J.A., Ross, M.L., Moore, D.R., Phillips, S.M., Slater, G.R., Stellingwerff, T., Tipton, K.D., Garnham, A.P., and Coffey, V.G. 2012. Preexercise aminoacidemia and muscle protein synthesis after resistance exercise. *Med Sci Sports Exerc,* 44**,** 1968-77.

266. Brook, M., Scaife, P., Bass, J., Cegielski, J., Watanabe, S., Wilkinson, D., Smith, K., Phillips, B., and Atherton, P. 2021. A collagen hydrolysate/milk protein-blend stimulates muscle anabolism equivalently to an isoenergetic milk protein-blend containing a greater quantity of essential amino acids in older men. *Clinical Nutrition,* 40**,** 4456-4464.

267. Elliot, T.A., Cree, M.G., Sanford, A.P., Wolfe, R.R., and Tipton, K.D. 2006. Milk ingestion stimulates net muscle protein synthesis following resistance exercise. *Medicine & Science in Sports & Exercise,* 38**,** 667-674.

268. Holwerda, A., Kouw, I., Trommelen, J., Halson, S., Wodzig, W., Verdijk, L., and van Loon, L.J.C. 2016. Physical Activity Performed in the Evening Increases the Overnight Muscle Protein Synthetic Response to Presleep Protein Ingestion in Older Men. 146**,** 1307-1314.

269. Yang, Y., Breen, L., Burd, N.A., Hector, A.J., Churchward-Venne, T.A., Josse, A.R., Tarnopolsky, M.A., and Phillips, S.M. 2012. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *British Journal of Nutrition,* 108**,** 1780-8.

270. Burd, N.A., Gorissen, S.H., and Van Loon, L.J. 2013. Anabolic resistance of muscle protein synthesis with aging. *Exercise and sport sciences reviews,* 41**,** 169-173.

271. Rennie, M.J. and Wilkes, E.A. 2005. Maintenance of the musculoskeletal mass by control of protein turnover: the concept of anabolic resistance and its relevance to the transplant recipient. *Annals of Transplantation,* 10**,** 31-4.

272. Phillips, S.M. 2016. The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutrition & Metabolism,* 13**,** 1-9.

273. Norton, L.E. and Layman, D.K. 2006. Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *The Journal of nutrition,* 136**,** 533S-537S.

274. Volpi, E., Campbell, W.W., Dwyer, J.T., Johnson, M.A., Jensen, G.L., Morley, J.E., and Wolfe, R.R. 2013. Is the Optimal Level of Protein Intake for Older Adults Greater Than the Recommended Dietary Allowance? *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 68**,** 677-681.

275. West, D.W., Burd, N.A., Coffey, V.G., Baker, S.K., Burke, L.M., Hawley, J.A., Moore, D.R., Stellingwerff, T., and Phillips, S.M. 2011. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *American Journal of Clinical Nutrition,* 94**,** 795-803.

276. Phillips, S.M. and Van Loon, L.J. 2011. Dietary protein for athletes: from requirements to optimum adaptation. *J Sports Sci,* 29 Suppl 1**,** S29-38.

277. Oikawa, S., Kamal, M., Webb, E., McGlory, C., Baker, S., and Phillips, S. 2020. Whey protein but not collagen peptides stimulate acute and longer-term muscle protein synthesis with and without resistance exercise in healthy older women: a randomized controlled trial. *Am J Clin Nutr,* 111**,** 708-718.

278. Beals, J.W., Skinner, S.K., McKenna, C.F., Poozhikunnel, E.G., Farooqi, S.A., van Vliet, S., Martinez, I.G., Ulanov, A.V., Li, Z., Paluska, S.A., and Burd, N.A. 2018. Altered anabolic signalling and reduced stimulation of myofibrillar protein synthesis after feeding and resistance exercise in people with obesity. *The Journal of Physiology,* 596**,** 5119-5133.

279. Symons, T.B., Sheffield-Moore, M., Mamerow, M.M., Wolfe, R.R., and Paddon-Jones, D. 2011. The anabolic response to resistance exercise and a protein-rich meal is not diminished by age. *Journal of Nutrition, Health & Aging,* 15**,** 376-81.

280. Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., and Group, P. 2009. Preferred reporting items for systematic reviews and meta-analyses. *PLoS Med,* 6**,** e1000097.

281. Dideriksen, K.J., Reitelseder, S., Malmgaard-Clausen, N., Bechshoeft, R., Petersen, R., Mikkelsen, U., and Holm, L. 2016. No effect of anti-inflammatory medication on postprandial and postexercise muscle protein synthesis in elderly men with slightly elevated systemic inflammation. *Experimental Gerontology,* 83**,** 120-129.

282. Mikkelsen, U.R., Dideriksen, K., Andersen, M.B., Boesen, A., Malmgaard-Clausen, N.M., Sørensen, I.J., Schjerling, P., Kjær, M., and Holm, L. 2015. Preserved skeletal muscle protein anabolic response to acute exercise and protein intake in well-treated rheumatoid arthritis patients. *Arthritis research & therapy,* 17**,** 1-19.

283. Agergaard, J., Bülow, J., Jensen, J., Reitelseder, S., Drummond, M., Schjerling, P., Scheike, T., Serena, A., and Holm, L. 2017. Light-load resistance exercise increases muscle protein synthesis and hypertrophy signaling in elderly men. *Am J Physiol Endocrinol Metab,* 312**,** E326-E338.

284. Areta, J.L., Burke, L.M., Camera, D.M., West, D.W.D., Crawshay, S., Moore, D.R., Stellingwerff, T., Phillips, S.M., Hawley, J.A., and Coffey, V.G. 2014. Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am J Physiol Endocrinol Metab,* 306**,** E989-E997.

285. McGlory, C., Wardle, S., Macnaughton, L.S., Witard, O.C., Scott, F., Dick, J., Bell, J.G., Phillips, S.M., Galloway, S.D., and Hamilton, D.L. 2016. Fish oil supplementation suppresses resistance exercise and feeding-induced increases in anabolic signaling without affecting myofibrillar protein synthesis in young men. *Physiological Reports,* 4.

286. Janssen, I. 2010. Evolution of sarcopenia research. *Applied Physiology, Nutrition, and Metabolism,* 35**,** 707-712.

287. Higgins, J.P., Altman, D.G., Gøtzsche, P.C., Jüni, P., Moher, D., Oxman, A.D., Savović, J., Schulz, K.F., Weeks, L., and Sterne, J.A. 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *Bmj,* 343**,** d5928.

288. Higgins, J.P., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., and Welch, V.A., *Cochrane handbook for systematic reviews of interventions*. 2019: John Wiley & Sons.

289. Atherton, P.J., Kumar, V., Selby, A.L., Rankin, D., Hildebrandt, W., Phillips, B.E., Williams, J.P., Hiscock, N., and Smith, K. 2017. Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clinical Nutrition,* 36**,** 888-895.

290. Bukhari, S., Phillips, B., Wilkinson, D., Limb, M., Rankin, D., Mitchell, W., Kobayashi, H., Greenhaff, P., Smith, K., and Atherton, P. 2015. Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. *Am J Physiol Endocrinol Metab,* 308**,** E1056-65.

291. Chan, A.H., D'Souza, R.F., Beals, J.W., Zeng, N., Prodhan, U., Fanning, A.C., Poppitt, S.D., Li, Z., Burd, N.A., and Cameron-Smith, D. 2019. The degree of Aminoacidemia after dairy protein ingestion does not modulate the postexercise anabolic response in young men: A randomized controlled trial. *The Journal of Nutrition,* 149**,** 1511-1522.

292. Churchward-Venne, T.A., Cotie, L.M., MacDonald, M.J., Mitchell, C.J., Prior, T., Baker, S.K., and Phillips, S.M. 2014. Citrulline does not enhance blood flow, microvascular circulation, or myofibrillar protein synthesis in elderly men at rest or following exercise. *American Journal of Physiology-Endocrinology and Metabolism,* 307**,** E71-E83.

293. Devries, M., McGlory, C., Bolster, D., Kamil, A., Rahn, M., Harkness, L., Baker, S., and Phillips, S. 2018. Protein leucine content is a determinant of shorter- and longer-term muscle protein synthetic responses at rest and following resistance exercise in healthy older women: a randomized, controlled trial. *Am J Clin Nutr,* 107**,** 217-226.

294. Devries, M., McGlory, C., Bolster, D., Kamil, A., Rahn, M., Harkness, L., Baker, S., and Phillips, S. 2018. Leucine, Not Total Protein, Content of a Supplement Is the Primary Determinant of Muscle Protein Anabolic Responses in Healthy Older Women. *The Journal of Nutrition,* 148**,** 1088-1095.

295. Dickinson, J., Gundermann, D., Walker, D., Reidy, P., Borack, M., Drummond, M., Arora, M., Volpi, E., and Rasmussen, B. 2014. Leucine-enriched amino acid ingestion after resistance exercise prolongs myofibrillar protein synthesis and amino acid transporter expression in older men. *The Journal of Nutrition,* 144**,** 1694-1702.

296. Fujita, S., Dreyer, H.C., Drummond, M.J., Glynn, E.L., Volpi, E., and Rasmussen, B.B. 2009. Essential amino acid and carbohydrate ingestion before resistance exercise does not enhance postexercise muscle protein synthesis. *J Appl Physiol,* 106**,** 1730-9.

297. Gwin, J.A., Church, D.D., Hatch-McChesney, A., Howard, E.E., Carrigan, C.T., Murphy, N.E., Wilson, M.A., Margolis, L.M., Carbone, J.W., Wolfe, R.R., Ferrando, A.A., and Pasiakos, S.M. 2021. Effects of high versus standard essential amino acid intakes on whole-body protein turnover and mixed muscle protein synthesis during energy deficit: A randomized, crossover study. *Clinical Nutrition,* 40**,** 767-777.

298. Hermans, W.J.H., Fuchs, C.J., Hendriks, F.K., Houben, L.H.P., Senden, J.M., Verdijk, L.B., and Van Loon, L.J.C. 2022. Cheese Ingestion Increases Muscle Protein Synthesis Rates Both at Rest and During Recovery from Exercise in Healthy, Young Males: a Randomized Parallel-Group Trial. *The Journal of Nutrition,* 152**,** 1022-1030.

299. Luiking, Y.C., Deutz, N.E., Memelink, R.G., Verlaan, S., and Wolfe, R.R. 2014. Postprandial muscle protein synthesis is higher after a high whey protein, leucine-enriched supplement than after a dairy-like product in healthy older people: a randomized controlled trial. *Nutrition Journal,* 13**,** 9.

300. Monteyne, A.J., Coelho, M.O.C., Porter, C., Abdelrahman, D.R., Jameson, T.S.O., Finnigan, T.J.A., Stephens, F.B., Dirks, M.L., and Wall, B.T. 2020. Branched-Chain Amino Acid Fortification Does Not Restore Muscle Protein Synthesis Rates following Ingestion of Lower- Compared with Higher-Dose Mycoprotein. *The Journal of Nutrition,* 150**,** 2931-2941.

301. Reitelseder, S., Dideriksen, K., Agergaard, J., Malmgaard-Clausen, N.M., Bechshoeft, R.L., Petersen, R.K., Serena, A., Mikkelsen, U.R., and Holm, L. 2019. Even effect of milk protein and carbohydrate intake but no further effect of heavy resistance exercise on myofibrillar protein synthesis in older men. *European Journal of Nutrition,* 58**,** 583-595.

302. West, D.W., Kujbida, G.W., Moore, D.R., Atherton, P.J., Burd, N.A., Padzik, J.P., De Lisio, M., Tang, J.E., Parise, G., Rennie, M.J., Baker, S.K., and Phillips, S.M. 2009. Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle

protein synthesis or intracellular signalling in young men. *Journal of Physiology,* 587**,** 5239-47.

303. Wilkinson, D., Bukhari, S., Phillips, B., Limb, M., Cegielski, J., Brook, M.S., Rankin, D., Mitchell, W., Kobayashi, H., Williams, J., Lund, J., Greenhaff, P.L., Smith, K., and Atherton, P.J. 2018. Effects of leucine-enriched essential amino acid and whey protein bolus dosing upon skeletal muscle protein synthesis at rest and after exercise in older women. *Clinical Nutrition,* 37**,** 2011-2021.

304. Dideriksen, K.J., Reitelseder, S., Petersen, S.G., Hjort, M., Helmark, I.C., Kjaer, M., and Holm, L. 2011. Stimulation of muscle protein synthesis by whey and caseinate ingestion after resistance exercise in elderly individuals. *Scandinavian Journal of Medicine & Science in Sports,* 21**,** e372-e383.

305. Phillips, S.M. and Van Loon, L.J. 2011. Dietary protein for athletes: from requirements to optimum adaptation. *Journal of Sports Sciences***,** 37-46.

306. Wall, B.T., Morton, J.P., and Van Loon, L.J.C. 2015. Strategies to maintain skeletal muscle mass in the injured athlete: Nutritional considerations and exercise mimetics. *European Journal of Sport Science,* 15**,** 53-62.

307. Collins, J., Maughan, R.J., Gleeson, M., Bilsborough, J., Jeukendrup, A., Morton, J.P., Phillips, S.M., Armstrong, L., Burke, L.M., Close, G.L., Duffield, R., Larson-Meyer, E., Louis, J., Medina, D., Meyer, F., Rollo, I., Sundgot-Borgen, J., Wall, B.T., Boullosa, B., Dupont, G., Lizarraga, A., Res, P., Bizzini, M., Castagna, C., Cowie, C.M., D'Hooghe, M., Geyer, H., Meyer, T., Papadimitriou, N., Vouillamoz, M., and McCall, A. 2021. UEFA expert group statement on nutrition in elite football. Current evidence to inform practical recommendations and guide future research. *British Journal of Sports Medicine,* 55**,** 416-416.

308. Morgan, P.T., Killer, S., Macnaughton, L.S., Dirks, M.L., Witard, O.C., Wall, B.T., and Breen, L. 2022. The BASES Expert Statement on protein recommendations for athletes: amount, type and timing. *The Sport and Exercise Scientist, British Association of Sport and Exercise Sciences*.

309. Dickinson, J.M., Volpi, E., and Rasmussen, B.B. 2013. Exercise and nutrition to target protein synthesis impairments in aging skeletal muscle. *Exercise and sport sciences reviews,* 41**,** 216.

310. Gorissen, S.H., Trommelen, J., Kouw, I.W., Holwerda, A.M., Pennings, B., Groen, B.B., Wall, B.T., Churchward-Venne, T.A., Horstman, A.M., and Koopman, R. 2020. Protein type, protein dose, and age modulate dietary protein digestion and phenylalanine absorption kinetics and plasma phenylalanine availability in humans. *The Journal of Nutrition,* 150**,** 2041-2050.

311. Milan, A., D'Souza, R., Pundir, S., Pileggi, C., Barnett, M., Markworth, J., Cameron-Smith, D., and Mitchell, C. 2015. Older adults have delayed amino acid absorption after a high protein mixed breakfast meal. *The journal of nutrition, health & aging,* 19**,** 839-845.

312. Boirie, Y., Gachon, P., and Beaufrere, B. 1997. Splanchnic and whole-body leucine kinetics in young and elderly men. *The American journal of clinical nutrition,* 65**,** 489-495.

313. Volpi, E., Mittendorfer, B., Wolf, S.E., and Wolfe, R.R. 1999. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *American Journal of Physiology-Endocrinology And Metabolism*.

314. Timmerman, K.L., Lee, J.L., Dreyer, H.C., Dhanani, S., Glynn, E.L., Fry, C.S., Drummond, M.J., Sheffield-Moore, M., Rasmussen, B.B., and Volpi, E. 2010. Insulin stimulates human skeletal muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation and mammalian target of rapamycin complex 1 signaling. *The Journal of Clinical Endocrinology & Metabolism,* 95**,** 3848-3857.

315. Norton, L.E., Layman, D.K., Bunpo, P., Anthony, T.G., Brana, D.V., and Garlick, P.J. 2009. The leucine content of a complete meal directs peak activation but not duration of skeletal muscle protein synthesis and mammalian target of rapamycin signaling in rats. *The Journal of nutrition,* 139**,** 1103-1109.

316. Mitchell, W.K., Phillips, B.E., Williams, J.P., Rankin, D., Lund, J.N., Wilkinson, D.J., Smith, K., and Atherton, P.J. 2015. The impact of delivery profile of essential amino acids upon skeletal muscle protein synthesis in older men: clinical efficacy of pulse vs. bolus supply. *American Journal of Physiology-Endocrinology and Metabolism,* 309**,** E450-E457.

317. Gorissen, S.H., Burd, N.A., Hamer, H.M., Gijsen, A.P., Groen, B.B.L., and Van Loon, L.J. 2014. Carbohydrate coingestion delays dietary protein digestion and absorption but does not modulate postprandial muscle protein accretion. *The Journal of Clinical Endocrinology & Metabolism,* 99**,** 2250-2258.

318. Staples, A.W., Burd, N.A., West, D., Currie, K.D., Atherton, P.J., Moore, D.R., Rennie, M.J., Macdonald, M.J., Baker, S.K., and Phillips, S.M. 2011. Carbohydrate does not augment exercise-induced protein accretion versus protein alone. *Med Sci Sports Exerc,* 43**,** 1154-61.

319. Koopman, R., Beelen, M., Stellingwerff, T., Pennings, B., Saris, W.H., Kies, A.K., Kuipers, H., and Van Loon, L.J. 2007. Coingestion of carbohydrate with protein does not further augment postexercise muscle protein synthesis. *American Journal of Physiology-Endocrinology and Metabolism,* 293**,** E833-E842.

320. Hamer, H.M., Wall, B.T., Kiskini, A., de Lange, A., Groen, B.B., Bakker, J.A., Gijsen, A.P., Verdijk, L.B., and van Loon, L.J. 2013. Carbohydrate co-ingestion with protein does not further augment post-prandial muscle protein accretion in older men. *Nutrition & Metabolism,* 10**,** 1-9.

321. Taylor, P.M. 2014. Role of amino acid transporters in amino acid sensing. *The American journal of clinical nutrition,* 99**,** 223S-230S.

322. Paulsen, G., Crameri, R., Benestad, H.B., Fjeld, J.G., Morkrid, L., Hallen, J., and Raastad, T. 2010. Time course of leukocyte accumulation in human muscle after eccentric exercise. *Medicine & Science in Sports & Exercise,* 42**,** 75-85.

323. Clarkson, P.M. and Hubal, M.J. 2001. Are women less susceptible to exercise-induced muscle damage? *Current opinion in clinical nutrition & metabolic care,* 4**,** 527-531.

324. Mihm, M., Gangooly, S., and Muttukrishna, S. 2011. The normal menstrual cycle in women. *Animal reproduction science,* 124**,** 229-236.

325. McNulty, K.L., Elliott-Sale, K.J., Dolan, E., Swinton, P.A., Ansdell, P., Goodall, S., Thomas, K., and Hicks, K.M. 2020. The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis. *Sports Medicine,* 50**,** 1813-1827.

326. Miller, B.F., Hansen, M., Olesen, J.L., Flyvbjerg, A., Schwarz, P., Babraj, J.A., Smith, K., Rennie, M.J., and Kjaer, M. 2006. No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism,* 290**,** E163-E168.

327. Hansen, M. and Kjaer, M. 2014. Influence of sex and estrogen on musculotendinous protein turnover at rest and after exercise. *Exercise and sport sciences reviews,* 42**,** 183-192.

328. Hansen, M., Langberg, H., Holm, L., Miller, B.F., Petersen, S.G., Doessing, S., Skovgaard, D., Trappe, T., and Kjaer, M. 2011. Effect of administration of oral contraceptives on the synthesis and breakdown of myofibrillar proteins in young women. *Scandinavian Journal of Medicine & Science in Sports,* 21**,** 62-72.

329. West, D.W.D., Burd, N.A., Churchward-Venne, T.A., Camera, D.M., Mitchell, C.J., Baker, S.K., Hawley, J.A., Coffey, V.G., and Phillips, S.M. 2012. Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *Journal of applied physiology (Bethesda, Md. : 1985),* 112**,** 1805-13.

330. Sayers, S.P. and Clarkson, P.M. 2001. Force recovery after eccentric exercise in males and females. *European Journal of Applied Physiology,* 84**,** 122-126.

331. Macintyre, D.L., Reid, W.D., Lyster, D.M., and McKenzie, D.C. 2000. Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. *European Journal of Applied Physiology and Occupational Physiology,* 81**,** 47-53.

332. Rinard, J., Clarkson, P.M., Smith, L.L., and Grossman, M. 2000. Response of males and females to high-force eccentric exercise. *Journal of Sports Sciences,* 18**,** 229-236.

333. Mackay, K., González, C., Zbinden-Foncea, H., and Peñailillo, L. 2019. Effects of oral contraceptive use on female sexual salivary hormones and indirect markers of muscle damage following eccentric cycling in women. *European Journal of Applied Physiology,* 119**,** 2733-2744.

334. Martínez-Gómez, M., Cruz, Y., Salas, M., Hudson, R., and Pacheco, P. 1994. Assessing pain threshold in the rat: changes with estrus and time of day. *Physiology & behavior,* 55**,** 651-657.

335. Romero-Parra, N., Cupeiro, R., Alfaro-Magallanes, V.M., Rael, B., Rubio-Arias, J.Á., Peinado, A.B., and Benito, P.J. 2021. Exercise-induced muscle damage during the menstrual cycle: a systematic review and meta-analysis. *The Journal of Strength & Conditioning Research,* 35**,** 549-561.

336. Janse, D.J.X., Thompson, B., and Han, A. 2019. Methodological recommendations for menstrual cycle research in sports and exercise. *Medicine and science in sports and exercise,* 51**,** 2610-2617.

337. Crameri, R.M., Aagaard, P., Qvortrup, K., Langberg, H., Olesen, J., and Kjaer, M. 2007. Myofibre damage in human skeletal muscle: effects of electrical stimulationversusvoluntary contraction. *The Journal of Physiology,* 583**,** 365-380.

338. Michailidis, Y., Karagounis, L.G., Terzis, G., Jamurtas, A.Z., Spengos, K., Tsoukas, D., Chatzinikolaou, A., Mandalidis, D., Stefanetti, R.J., Papassotiriou, I., Athanasopoulos, S., Hawley, J.A., Russell, A.P., and Fatouros, I.G. 2013. Thiol-based antioxidant supplementation alters human skeletal muscle signaling and attenuates its inflammatory response and recovery after intense eccentric exercise. *The American Journal of Clinical Nutrition,* 98**,** 233-245.

339. Warren, G.L., Lowe, D.A., and Armstrong, R.B. 1999. Measurement Tools Used in the Study of Eccentric Contraction Induced Injury. *Sports Medicine,* 27**,** 43-59.

340. Bowtell, J.L., Sumners, D.P., Dyer, A., Fox, P., and Mileva, K.N. 2011. Montmorency Cherry Juice Reduces Muscle Damage Caused by Intensive Strength Exercise. *Medicine & Science in Sports & Exercise,* 43**,** 1544-1551.

341. Machin, D.R., Christmas, K.M., Chou, T.-H., Hill, S.C., Van Pelt, D.W., Trombold, J.R., and Coyle, E.F. 2014. Effects of differing dosages of pomegranate juice supplementation after eccentric exercise. *Physiology Journal,* 2014.

342. Peake, J., Nosaka, K.K., and Suzuki, K. 2005. Characterization of inflammatory responses to eccentric exercise in humans.

343. Yu, J.G., Furst, D.O., and Thornell, L.E. 2003. The mode of myofibril remodelling in human skeletal muscle affected by DOMS induced by eccentric contractions. *Histochem Cell Biol,* 119**,** 383-93.

344. Carlsson, L., Yu, J.-G., Moza, M., Carpén, O., and Thornell, L.-E. 2007. Myotilin–a prominent marker of myofibrillar remodelling. *Neuromuscular Disorders,* 17**,** 61-68.

345. Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., and Wood, A. 2019. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *The lancet,* 393**,** 447-492.

346. Chakrabarti, S., Jahandideh, F., and Wu, J. 2014. Food-Derived Bioactive Peptides on Inflammation and Oxidative Stress. *BioMed Research International,* 2014**,** 1-11.

347. Miles, M.P., Pearson, S.D., Andring, J.M., Kidd, J.R., and Volpe, S.L. 2007. Effect of Carbohydrate Intake during Recovery from Eccentric Exercise on Interleukin-6 and Muscle-Damage Markers. *International Journal of Sport Nutrition and Exercise Metabolism,* 17**,** 507-520.

348. Cuthbertson, D.J., Babraj, J., Smith, K., Wilkes, E., Fedele, M.J., Esser, K., and Rennie, M. 2006. Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise. *Am J Physiol Endocrinol Metab,* 290**,** E731-8.

349. Børsheim, E., Aarsland, A., and Wolfe, R.R. 2004. Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. *International journal of sport nutrition and exercise metabolism,* 14**,** 255-271.

350. D'Hulst, G., Masschelein, E., and De Bock, K. 2022. Resistance exercise enhances long-term mTORC1 sensitivity to leucine. *Molecular Metabolism,* 66**,** 101615.

351. Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T.L., Maclean, D.A., and Pedersen, B.K. 1997. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *The Journal of Physiology,* 499**,** 833-841.

352. Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S., and Nimmo, M.A. 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nature Reviews Immunology,* 11**,** 607-615.

353. Moller-Loswick, A., Zachrisson, H., Hyltander, A., Korner, U., Matthews, D., and Lundholm, K. 1994. Insulin selectively attenuates breakdown of nonmyofibrillar proteins in peripheral tissues of normal men. *American Journal of Physiology-Endocrinology and Metabolism,* 266**,** E645-E652.

354. Paulsen, G., BENESTAD, H.B., STRØM-GUNDERSEN, I., MØRKRID, L., LAPPEGÅRD, K.T., and Raastad, T. 2005. Delayed leukocytosis and cytokine response to high-force eccentric exercise. *Medicine & Science in Sports & Exercise,* 37**,** 1877-1883.

355. Willoughby, D.S., McFarlin, B., and Bois, C. 2003. Interleukin-6 Expression After Repeated Bouts of Eccentric Exercise. *International Journal of Sports Medicine,* 24**,** 15-21.

356. Dumont, N.A. and Frenette, J. 2013. Macrophage Colony-Stimulating Factor–Induced Macrophage Differentiation Promotes Regrowth in Atrophied Skeletal Muscles and C2C12 Myotubes. *The American Journal of Pathology,* 182**,** 505-515.

357. Rodenburg, J., De Boer, R., Schiereck, P., Van Echteld, C., and Bär, P. 1994. Changes in phosphorus compounds and water content in skeletal muscle due to eccentric exercise. *European journal of applied physiology and occupational physiology,* 68**,** 205-213.

358. Tischler, M.E., Desautels, M., and Goldberg, A.L. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? *Journal of Biological Chemistry,* 257**,** 1613-1621.

359. Wall, B.T., Dirks, M.L., Snijders, T., van Dijk, J.W., Fritsch, M., Verdijk, L.B., and van Loon, L.J. 2016. Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion. *American Journal of Physiology - Endocrinology & Metabolism,* 310**,** E137-47.

360. Kilroe, S.P., Fulford, J., Jackman, S.R., Holwerda, A., Gijsen, A.P., van Loon, L.J.C., and Wall, B.T. 2020. Dietary protein intake does not modulate daily myofibrillar protein synthesis rates or loss of muscle mass and function during short-term immobilization in young men: a randomized controlled trial. *American Journal of Clinical Nutrition*.

361. Yu, J.G., Liu, J.X., Carlsson, L., Thornell, L.E., and Stal, P.S. 2013. Re-evaluation of sarcolemma injury and muscle swelling in human skeletal muscles after eccentric exercise. *PLoS One,* 8**,** e62056.

362. Hinks, A., Hawke, T.J., Franchi, M.V., and Power, G.A. 2023. The importance of serial sarcomere addition for muscle function and the impact of aging. *Journal of Applied Physiology*.

363. Roman, W., Pinheiro, H., Pimentel, M.R., Segalés, J., Oliveira, L.M., García-Domínguez, E., Gómez-Cabrera, M.C., Serrano, A.L., Gomes, E.R., and Muñoz-Cánoves, P. 2021. Muscle repair after physiological damage relies on nuclear migration for cellular reconstruction. *Science,* 374**,** 355-359.

364. Dos Santos, L., Cyrino, E.S., Antunes, M., Santos, D.A., and Sardinha, L.B. 2017. Sarcopenia and physical independence in older adults: the independent and synergic role of muscle mass and muscle function. *Journal of Cachexia, Sarcopenia and Muscle,* 8**,** 245-250.

365. Reeves, N.D., Maganaris, C.N., Longo, S., and Narici, M.V. 2009. Differential adaptations to eccentric versus conventional resistance training in older humans. *Experimental Physiology,* 94**,** 825-833.

366. Lee, S.K. 2018. Sex as an important biological variable in biomedical research. *BMB Reports,* 51**,** 167-173.

367. Biolo, G., Fleming, R.D., Maggi, S.P., Nguyen, T.T., Herndon, D.N., and Wolfe, R.R. 2002. Inverse regulation of protein turnover and amino acid transport in skeletal muscle of hypercatabolic patients. *The Journal of Clinical Endocrinology & Metabolism,* 87**,** 3378-3384.

368. Tarnopolsky, M.A., MacDougall, J.D., and Atkinson, S.A. 1988. Influence of protein intake and training status on nitrogen balance and lean body mass. *Journal of Applied Physiology,* 64**,** 187-193.

369. Phillips, S.M., Chevalier, S., and Leidy, H.J. 2016. Protein “requirements” beyond the RDA: implications for optimizing health. *Applied Physiology, Nutrition, and Metabolism,* 41**,** 565-572.

9 Appendix

Table 9.1 Characterisation of exercise induced muscle damage utilising transmission electron microscopy imaging.

	Characterisation	Categorisation			
Raastad, 2009	Sarcomere disruption was characterised as myofilament disorganisation and loss of Z disk integrity At least 10 fibres analysed per subject	Focal: less than 3 sarcomeres in both directions affected	Moderate: 3-9 sarcomeres affected	Extreme: >10 sarcomeres both directions	
Newham 1983,	Number of fibres with myofibrillar disruption (% of total counted) 30-90 individual fibres per biopsy	Focal: areas of disruption affecting one or two adjacent myofibrillar and one or two adjacent sarcomeres	Extensive: disruption affecting more than two adjacent sarcomeres and more than two adjacent myofibrils or more than ten focal areas, damage	Very extensive: fibres which contained more than one extensive area of damage	
Yu, 2004	The percentage of muscle fibres showing any alterations in the transverse band pattern The number of fibres with any type of change were counted. If a fibre had more than one type of change,	Amorphous widened Z-discs: Z-discs with irregular and amorphous shapes and decreased electron density	Double Z-discs: material of the electron density of Z-discs that are present as double rows of dots or lines at the location of a Z-disc and are connected with material of electron density intermediate between that	Amorphous sarcomeres: characterised by a disturbed pattern of one or more sarcomere lengths, lacking normal Z-discs	Supernumerary sarcomeres: areas with additional sarcomeres and containing dense longitudinal strands

	the fibre was counted repeatedly		of the I-bands and the Z-discs	
Crameri, 2007	A simple, random sample of 10 digitized electron microscopy images was obtained from each biopsy. On average, 1687 ± 44 Z-lines were counted per biopsy sample. Percentage of each was calculated.	Intact	Disrupted: Z lines still present but showing morphological changes from the intact Z lines	Destroyed: Z lines not intact
Friden, 1981	At the ultrastructural level the disturbances were found to originate from the myofibrillar Z-band which showed a marked broadening, streaming and, at places, total disruption	Focal disturbances	Myofilamentous material in sarcomeres adjacent to the affected Z bands were either super contracted or disorganised and out of register. Z bands were seen to have gaps in their lattice pattern	Z-bands during overloading constitute a weak link in the myofibrillar contractile chain. A disruption of myofibrils may result in a formation of protein components and subsequent releasing of protein bound ions
Friden, 1983	Determination of relative area occupied was point counted	Myofibrillar changes involving at least one sarcomere and total dispersing of the myofibrillar filament within many sarcomeres were encircled		

Figure 9.1 Example meal plan and compliance log

Friday 8th November 2019

Please write the time you consumed each ingredient and if anything was left over. Please return this form to the researchers.

Breakfast	Time of consumption	Left over
Quaker oats		
Orange juice		
Supplement		

Lunch	Time of consumption	Left over
Bread (3 slices)		
Ham (2 slices)		
Tomato		
Mayonnaise		
Jam		

Dinner	Time of consumption	Left over
Wraps (2)		
Chicken breast		
Peppers		
Salsa		
Sour cream		
Olive oil		
Fajita spice mix		
Chocolate pudding pot		

Snacks	Time of consumption	Left over
Banana		
Apple		
Strawberry Muller corner		
Walkers crisps		
Apple fruity bake		

Table 9.2 Risk of Bias assessment

	Selection bias		Performance and detection bias	Attrition bias	Reporting bias	Other bias
	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?	Free of other bias?
Agergaard, 2017						
Areta, 2014						
Atherton, 2017						
Beals, 2018						
Borack, 2016						
Brook, 2021						
Bukhari, 2015						
Burd, 2015						
Burd, 2010						
Burd, 2012						
Burd, 2012						
Chan, 2019						
Churchward-Venne, 2014						
Churchward-Venne, 2014						
Devries, 2018						
Devries, 2018						
Dickinson, 2014						
Dideriksen, 2016						
Dreyer, 2008						
Fujita, 2009						
Gwin, 2021						
Hermans, 2022						
Hermans, 2022						
Luiking, 2014						
McGlory, 2016						
McKendry, 2016						
Mikkelsen, 2015						
Monteyne, 2020						
Monteyne, 2020						
Moore, 2009						
Oikawa, 2020						
Pinckaers, 2022						
Reidy, 2013						
Reitelseder, 2019						
Symons, 2011						
van Vliet, 2017						
West, 2009						
Wilkinson, 2018						

	Low risk of bias
	High risk of bias
	Unclear risk of bias

Table 9.3 Systematic review summary table- predetermined relevant outcome variables from each study were extracted in relation to study characterisation and outcomes measures. Further raw data which can be accessed via supplementary information online.

Author	Study design	*n*	Age	Participant characteristics	Exercise protocol	Protein/ amino acid source	Protein dose (g)	Leucine dose (g)	AA Fortification	Coingestion	Basal MPS (%/h)
Agergaard, 2017	Double blinded, parallel RCT	10	69.7 ±4.7	Healthy elderly men	10 x36 @16% 1RM unilateral leg extension	Whey protein	40.4	3.2	N	N	0.052
Areta, 2014	Crossover RCT	15	27 ±5 28 ±4	Young healthy resistance trained subjects (8M, 7F)	6 x8 @80%1RM bilateral leg press	Whey protein	15.0	1.4	N	N	0.027
							30.0	2.7	N	N	0.027
Atherton, 2017	Parallel RCT	9	24 ±6	Healthy young males	6 x8 @ 75%1RM isotonic unilateral leg extension	Meal replacement	10.0	5.2	Y-Leucine 4.2g	N	0.042
		9	24 ±6	Healthy young males		Meal replacement	10.0	1.0	Y- Alanine 4.2g	N	0.037
		9	70 ±5	Healthy old males		Meal replacement	10.0	5.2	Y-Leucine 4.2g	N	0.042
		9	70 ±5	Healthy old males		Meal replacement	10.0	1.0	Y- Alanine 4.2g	N	0.039
Beals, 2018	Parallel RCT	9	21 ±1	Normal weight, healthy, not involved in regular exercise (5M, 4F)	4 x10-12 @ 75%1RM unilateral leg extension	Lean ground pork	36.0	3.3	N	N	0.060
		9	22 ±1	Obese, healthy, not involved in		Lean ground pork	36.0	3.3			0.050

				regular exercise (5M, 4F)							
Borack, 2016	Double blinded, parallel RCT	10	69.3 ±2.1	Healthy men	8 x10 @70%1RM bilateral leg extension	Whey protein	30.4	3.3	N	N	0.054
		9	62.2 ±1.5			Soy dairy protein blend	30.5	2.7			0.074
Brook, 2021	Blinded, crossover RCT	8	71 ±1	Older males	6 x8 @75%1RM unilateral knee extension	Collagen protein hydrolysates + milk protein	20.0	0.9	N	N	0.042
		8				Milk protein	20.0	1.9	N	N	0.039
Bukhari, 2015	Parallel RCT	8	66 ±1	Healthy older post-menopausal women	6x8 @75%1RM unilateral knee extension	Whey protein	20.0	2.0	N	N	0.066
		8	66 ±1			Leucine enriched amino acids	3.0	1.2	Y	N	0.071
Burd, 2015	Crossover RCT	12	22 ±1	Healthy young men, recreationally active	4 x8-10 to volitional fatigue bilateral leg press and leg extension	Non -fat skim milk	30.0	2.7	N	N	0.032
		12				Minced beef	30.0	2.5	N	N	0.033
Burd, 2010	Parallel RCT	8	24.3 ±1.6	Recreationally resistance trained males (at least 1 session per week)	1 set @70%1RM until volitional exhaustion unilateral leg extension	Whey protein isolate	20.0	2.8	N	N	0.029

		8	24.3 ±1.6		3 x @70%1RM until volitional exhaustion unilateral leg extension	Whey protein isolate	20.0	2.8	N	N	0.029
Burd, 2012	Parallel RCT	7	72 ±1	Healthy older men light to moderately active	3 x10 @10RM unilateral knee extension	Micellar casein	20.0	1.6	N	N	0.021
		7	72 ±1			Whey protein isolate	20.0	2.8	N	N	0.021
Burd, 2012	Parallel RCT	8	23.5 ±1	Resistance trained men	3 x 30%1RM until failure, slow (6s concentric phase and a 6s eccentric phase) unilateral leg extension	Whey protein isolate	20.0	2.6	N	N	0.020
		8	23.5 ±1		3 x 30%1RM matched at an equivalent load to slow condition (1s concentric phase and a 1s eccentric phase) unilateral leg extension	Whey protein isolate	20.0	2.6	N	N	0.020

Chan, 2019	Double blinded, parallel RCT	10	23.7 ±3.3	Healthy young men with no lower body resistance training	3 x10 @80%1RM bilateral leg press and leg extension (final set to exhaustion)	Milk protein concentrate	25.0	2.6	N	N	0.036
		10	21.3 ±2.1			Mineral modified milk protein concentrate	25.0	2.6	N	N	0.028
		10	22.7 ±3.2			Calcium Caseinate	25.0	2.4	N	N	0.035
Churchward-Venne, 2014	Double blinded, parallel RCT	8	20.9 ±0.6	Young males	8 x10-12 @80%1RM unilateral knee extension	Whey protein	25.0	3.0	N	Y-22.6g CHO and 5.68g Fat	0.018
		8	20.5 ±1.1			Whey protein	6.3	0.8	N	Y-35g CHO and 5.68g Fat	0.022
		8	20.4 ±0.6			Whey protein	6.3	3.0	Y -Leucine 2.25g	Y-35g CHO and 5.68g Fat	0.019
		8	19.5 ±0.1			Whey protein	6.3	5.0	Y-Leucine 4.25g	Y-35g CHO and 5.68g Fat	0.020
		8	20.8 ±0.8			Whey protein	6.3	5.0	Y- Leucine 4.25g, isoleucine 1.01g, valine 1.03g	Y-35g CHO and 5.68g Fat	0.018
Churchward-Venne, 2014	Blinded, parallel RCT	7	72.4 ±1.9	Independently living healthy elderly men	6 x8-10 @80%10RM unilateral	Whey protein	45.0	5.4	N	N	0.015
		7	73.7 ±1.3			Whey protein	15.0	1.8	Y -10g Citrulline	N	0.014

		7	71.9 ±2.0		seated knee extension	Whey protein	15.0	1.8	Y -NEAA	N	0.015
Devries, 2018	Single blinded, parallel RCT	11	68 ±1	Healthy older women	4x (2x) 50%, (2x) 60%1RM unilateral knee extension	Whey protein isolate	24.9	2.9	N	N	0.035
		11	69 ±1			Milk protein (milk protein concentrate and milk protein isolate)	10.3	3.0	Y -2.6g leucine	N	0.034
Devries, 2018	Single blinded, parallel RCT	11	69 ±1	Healthy older women	4x (2x) 50%, (2x) 60%1RM unilateral knee extension	Milk protein (milk protein concentrate and milk protein isolate)	15.0	4.2	Y -3.1g leucine	N	0.034
		11	68 ±1			Blended protein (milk protein, milk protein isolate, soy protein)	15.0	1.3	N	N	0.034
Dickinson, 2014	Parallel RCT	7	74 ±2	Healthy older men	8 x10 @65%1RM bilateral leg extension	EAA	10.0	1.9	N	N	0.050
		8	71 ±3			EAA	10.0	3.5	N	N	0.048

Dideriksen, 2016	Parallel RCT	10	69 ±2	Elderly healthy males	10 x 8@70%1RM unilateral knee extension	Whey protein isolate	25.6	2.8	N	N	0.030
Dreyer, 2008	Parallel RCT	16	27 ±2	Young healthy males, not currently engaged in resistance exercise	10 x10 @70%1RM bilateral leg extension exercise	EAA	21.7	1.7	Y -leucine enriched EAA nutrient solution	Y- 22g sucrose	0.065
Fujita, 2009	Parallel RCT	11	25 ±1	Young healthy individuals (6M, 5F)	10 x10@70%1RM bilateral leg extension	EAA	18.4	6.4	Y -leucine enriched EAA nutrient solution	Y- 25.5g sucrose	0.064
Gwin, 2021	Double blinded, crossover RCT	19	23 ±5	Healthy young non obese resistance trained males	8 x 10@80%1RM unilateral leg press and leg extension	EAA	8.0	2.8	N	N	0.041
						EAA	23.9	8.4	N	N	0.040
Hermans, 2022	Parallel RCT	10	25 ±4	Healthy young males	4 x8-10 @80% 1RM unilateral leg press and leg extension (last set to volitional fatigue)	Cheese	30.0	2.4	N	N	0.031
		10				Milk protein concentrate	30.0	2.6	N	N	0.030
Hermans, 2022	Parallel RCT	11	24 ±3	Healthy young men	5 x8-10 @80% 1RM unilateral leg press and leg extension	Lesser mealworm derived protein	30.0	2.5	N	N	0.025

		12	22 ±3		(last set to volitional fatigue)	Milk protein	30.0	2.8	N	N	0.026
Luiking, 2014	Double blinded, parallel RCT	9	66.9 ±4.8	Healthy older adults (4M, 5F)	4 x8-10 @80%1RM unilateral leg extension	Whey protein	21.0	3.0	Y- leucine enriched	N	0.053
		10	71.1 ±6.3	Healthy older adults (5M, 5F)		Milk protein	6.0	0.6	N	N	0.052
McGlory, 2016	Parallel RCT	10	24 ±0	Resistance trained males	3 x10 @70%1RM unilateral leg press and leg extension	Whey protein	30.0	2.2	N	N	0.025
		9	21 ±0			Whey protein	30.0	2.2	N	N	0.024
McKendry, 2016	Parallel RCT	8	24.5 ±4.8	Young males, recreationally active (lower limb resistance training at least once per week for 1year)	4 x 75%1RM bilateral leg press and leg extension until fatigue (1min rest)	Whey protein isolate	25.0	2.4	N	N	0.025
		8	23.7 ±5.4		4 x 75%1RM bilateral leg press and leg extension until fatigue (5min rest)	Whey protein isolate	25.0	2.4	N	N	0.028
Mikkelsen, 2015	Parallel RCT	13	57 ±15	Healthy controls	10 x8@70%1RM unilateral knee extension	Whey protein isolate	25.7	2.84	N	N	0.035
		10	22 ±1			Mycoprotein	31.5	2.5	N	N	0.025

Monteyne, 2020	Double blinded, parallel RCT	9	22 ±1	Young healthy men, recreationally active and experienced with resistance exercise >3times/wk	5 x30 unilateral maximal concentric isokinetic leg extension and leg flexion contractions	Mycoprotein	18.7	2.5	Y- enriched BCAA	N	0.031
Monteyne, 2020	Double blinded, parallel RCT	10	22 ±1	Young healthy men, recreationally active and experienced with resistance exercise >3times/wk	5 x30 unilateral maximal concentric isokinetic leg extension and leg flexion contractions	Milk protein	26.2	2.5	N	N	0.036
Moore, 2009	Parallel RCT	7	26 ±3	Healthy, recreationally active males	5 x8-10 unilateral leg press and knee extension until voluntary failure	Whey protein	25.0	1.8	N	N	0.025
Oikawa, 2020	Double blinded, parallel RCT	11	67 ±2	Healthy older women	4x8-10 @60%1RM unilateral dominant leg knee extension	Whey protein isolate	30.0	4.3	N	N	0.020
		11	69 ±4			Hydrolysed collagen	30.0	0.9	N	N	0.022
Pinckaers, 2022	Double blinded, parallel RCT	12	23 ±3	Healthy recreationally active males	4 x 8@80% 1RM unilateral leg press and leg extension (4th	Potato protein	30.0	2.6	N	N	0.022
		12	25 ±4			Milk protein	30.0	2.6	N	N	0.022

					set as many reps as possible)						
Reidy, 2013	Double blinded, parallel RCT	10	23.1 ±1.0	Healthy young recreationally active participants (17M, 2F)	8x10 @55,60, 65, 70% (70% sets 4-8)1RM bilateral leg extension	Protein Blend (50% sodium caseinate, 25% whey protein isolate, 25% soy protein isolate)	19.3	1.8	N	N	0.057
		9	25.1 ±1.2			Whey protein isolate	17.7	1.9	N	N	0.058
Reitelseder, 2019	Single blinded, parallel RCT	10	69 ±2	Moderately active older men	10x 8@70%1RM unilateral leg extension	Whey hydrolysate	27.2	2.3	N	N	0.027
		9	68 ±2			Caseinate	26.1	2.5	N	N	0.030
Symons, 2011	Parallel RCT	7	29 ±3	Healthy physically active and independent younger adults (3M, 4F)	6 x 8@80%1RM bilateral leg extensions	90% lean ground beef	90.0	5.9	N	N	0.074
		8	67 ±2	Healthy physically active and independent older adults (3M, 4F)		90% lean ground beef	90.0	5.9	N	N	0.075
		10	21 ±1			Whole egg	18.0	1.6	N	N	0.015

van Vliet, 2017	Crossover RCT	10	21 ±1	Healthy young men, regular structured resistance exercise training	4x 10 @80% 10RM bilateral leg press and leg extension	Egg white	18.0	1.6	N	N	0.015
West, 2009	Crossover RCT	8	20 ±1.1	Healthy young recreationally active men	4x 10@90% 10RM unilateral arm cable curl	Whey protein	25.0	2.4	N	N	0.039
		8	20 ±1.1		4x10 @90% 10RM unilateral arm cable curl, 5x10 @90% 10RM leg press and 3 x12 @90%12RM leg extension and leg curl superset	Whey protein	25.0	2.4	N	N	0.039
Wilkinson, 2018	Parallel RCT	8	65 ±1	Recreationally active, healthy older, post-menopausal women	6x 8@75% 1RM unilateral knee extension	EAA	1.5	0.6	Y-leucine enriched	N	0.061
		8	63 ±1			EAA	6.0	2.4	Y-leucine enriched	N	0.066
		8	66 ±1			Whey protein	40.0	4.0	N	N	0.056

Figure 9.2 Delta change (postprandial post-exercise increase) in muscle protein synthesis rates (MPS), expressed as fractional synthetic rate (FSR), in response to protein dose (g) in all participants (77 study arms) (**A**), young participants (45 study arms; 19-29 years) (**B**) and older participants (32 study arms; 57-74years) (**C**). Data were analysed by linear regression; coefficient of determination (r2), *P* value and y-intercept (b0) are presented