Management strategies and contributory factors for resistance exercise-induced muscle damage: an exploration of dietary protein, exercise load, and sex

PEARSON, ALICE,GRACE

Management strategies and contributory factors for resistance exercise-induced muscle damage: an exploration of dietary protein, exercise load, and sex

Alice Grace Pearson

A thesis submitted for the degree of

Doctor of Philosophy

Department of Sport and Exercise Sciences

Durham University

July 2023

DECLARATION

I confirm that the work in this thesis is my own, conducted under the supervision of Dr Karen Hind and Dr Lindsay Macnaughton, within the Department of Sport and Exercise Sciences, Durham University, United Kingdom. No part of this thesis has been submitted elsewhere for any other degree or qualification.

STATEMENT OF COPYRIGHT

ABSTRACT

The World Health Organisation recommends that resistance exercise be performed at least twice per week to benefit general health and wellbeing. However, resistance exercise is associated with acute muscle damage that potentially can dampen muscle adaptations promoted by chronic resistance training. The extent to which muscle is damaged by exercise is influenced by various factors, including age, training status, exercise type, and – notable to this thesis – sex. To this end, establishing sex-specific management strategies for exercise-induced muscle damage (EIMD) is important to optimise the benefits of exercise. Two EIMD management strategies were focussed on in this thesis: dietary protein supplementation and exercise load manipulation.

It was identified in this thesis that research into the impact both of protein supplementation and exercise load on EIMD heavily underrepresent female populations (*chapters 3 and 5*), despite well-documented sex differences in EIMD responses. Therefore, future research priority should be placed on bridging the sex data gap by conducting high-quality studies centralising around female-focussed and sex-comparative methodological designs.

Both peri-exercise protein supplementation and exercise load manipulation in favour of lighter loads were revealed to be effective management strategies for resistance EIMD in males through systematic and scoping review of the current literature (*chapters 3 and 5*, respectively). Due to a lack of data from females, it is only appropriate for these strategies to be recommended for males at present. To decipher whether protein supplementation and lower exercise loads are beneficial for managing EIMD in females, a randomised controlled trial (RCT) (*chapter 4*) and a protocol for an RCT (*chapter 6*) involving male and female participants are presented in this thesis.

The incorporation of ecologically-valid resistance exercise in the RCT in *chapter 4* highlighted that even mild muscle damage is attenuated in females, reflected in diminished increases in post-exercise creatine kinase concentration and muscle soreness compared with males; however, the reason for this difference requires further investigation. This study, while supporting sex differences, contrasted previous studies, as neither males nor females experienced an attenuation of EIMD during milk protein supplementation. This difference likely owed to the lower severity of muscle damage induced in the current study relative to previous studies, and accordingly, future research should seek to discover alternative management strategies for mild EIMD. A protocol for an RCT examining the impact of

exercise load on EIMD in untrained males and females is described in *Chapter 6* of this thesis and may be used as guidance for researchers developing similar, sex-comparative studies. It was hypothesised that females will experience attenuated muscle damage relative to males and low-load exercise will induce less muscle damage than high-load exercise in both sexes.

A lack of methodological consistency among EIMD studies was a recurring finding throughout this thesis, which posed an issue when attempting to compare between-study outcomes and reach a consensus. Achieving greater uniformity in study designs by adopting comparable methods relating to EIMD markers and time-points of assessment would help improve understanding of the factors influencing the magnitude of EIMD and effective management strategies. While there are limitations with several EIMD markers – for example the variability of biomarkers and subjectivity of perceptual assessments – once the optimal markers are determined, these should be consistently used moving forward.

Overall, this thesis has contributed to the current body of knowledge by demonstrating that milk protein ingestion is not an effective management strategy for muscle damage following ecologically-valid resistance exercise; therefore, alternative strategies to mitigate mild muscle damage should be investigated. Further, this work supported previous reports of sex differences in EIMD and indicated that the attenuation of EIMD in females relative to males was not attributed to sex differences in body composition; thus, the aetiology of such differences necessitates further exploration by means of high-quality sex comparative research. Finally, this thesis reached the consensus recommendation that lower exercise loads can be utilised to reduce muscle damage in males; nonetheless, supporting evidence for the application of this recommendation to females is required.

ACKNOWLEDGEMENTS

This PhD thesis is dedicated to Professor Kevin Tipton who, in the short time as my mentor, taught me so very much.

I would like to thank my supervisors, Karen and Lindsay, for guiding me through this process; my dear friend, Lauren, for being my emotional rock; and Oliver for his unconditional support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS

ATP	Adenosine triphosphate
BF%	Percent body fat
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMI	Body mass index
CHO	Carbohydrate
CK	Creatine kinase
[CK]	Creatine kinase concentration
CON	Control supplement group
CV	Coefficient of variation
DXA	Dual-energy X-ray absorptiometry
E-C	Excitation-contraction
EIMD	Exercise-induced muscle damage
ELISA	Enzyme-linked immunosorbent assay
ER	Oestrogen receptors
ES	Effect size
FFM	Fat-free mass
FSH	Follicle stimulating hormone
HC	Hormonal contraceptive
HL	High-load
HRT	Hormone replacement therapy
ICC	Intraclass correlation coefficient
LBM	Lean body mass
LDH	Lactate dehydrogenase
LH	Luteinising hormone
LL	Low-load
MAPK	Mitogen-activated protein kinases
Mb	Myoglobin
MC	Menstrual cycle
MHC	Myosin heavy chain
MILK-PRO	Milk protein supplement group

MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mTORC1	Mechanistic target of rapamycin complex 1
OCP	Oral contraceptive pill
PEDro	Physiotherapy evidence database
PLA_2	Phospholipase A_2
PPT	Pressure-pain threshold
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PRO	Protein
RBE	Repeated bout effect
RCT	Randomised controlled trial
RM	Repetition maximum
RNS	Reactive nitrogen species
ROM	Range of motion
ROS	Reactive oxygen species
RyR	Ryanodine receptor
SD	Standard deviation
VAS	Visual analogue scale
VRS	Visual rating scale

LIST OF PUBLICATIONS

Publications derived from this PhD work:

Pearson, A.G., Macnaughton, L.S., Hind, K. (2023). Milk Protein Ingestion does not Enhance Recovery from Muscle-Damaging Resistance Exercise in Untrained Males and Females: A Randomised Controlled Trial. *Applied Physiology, Nutrition, and Metabolism*. https://doi.org/10.1139/apnm-2022-0385

Pearson, A.G., Hind, K. & Macnaughton, L.S. (2022). The impact of dietary protein supplementation on recovery from resistance exercise-induced muscle damage: A systematic review with meta-analysis. *European Journal of Clinical Nutrition*. https://doi.org/10.1038/s41430-022-01250-y

Pearson, A.G., Macnaughton, L.S., & Hind, K. (2022). Sex differences in the impact of resistance exercise load on muscle damage: A protocol for a randomised parallel group trial. *PloS one*, *17*(9), e0275221. https://doi.org/10.1371/journal.pone.0275221

Additional publications achieved during the course of this PhD:

Pearson, A.G., Alexander, L., Witard, O.C., Coughlin, T.E., Tipton, K.D., Walshe, I.H (2021). A hypoenergetic diet with decreased protein intake does not reduce lean body mass in trained females. *European Journal of Applied Physiology*, 121, 771–781. https://doi.org/10.1007/s00421-020-04555-7

Jones, W., **Pearson, A**., Glassbrook, D., Slater, D., Dodd-Reynolds, C., Hind, K. (2022). Precision of the GE Lunar Total Body-Less Head Scan for the Measurement of Three-Compartment Body Composition in Athletes. *Journal of Clinical Densitometry*, *25*(4), 692-698. https://doi.org/10.1016/j.jocd.2022.08.008

Peer-reviewed conference presentations:

Pearson, A.G., Hughes, A., Witard, O.C. A muscle-centric view of plant proteins for young adults. Meeting Future Global Protein Requirements, *Rank Prize*. Oral presentation (October 2022) (Rank Prize 1st place award for oral presentation)

Pearson, A.G., Macnaughton, L.S., Hind, K. The impact of milk protein ingestion on resistance exercise-induced muscle damage in untrained males and females. Fifth annual early

career researcher conference, *Wolfson Research Institute*. Three-minute flash talk presentation (June 2022) (Wolfson Research Institute 1st place award for flash presentation)

Pearson, A.G., Alexander, L., Witard, O.C., Coughlin, T.E., Tipton, K.D., Walshe, I.H. A hypoenergetic diet with decreased protein intake does not reduce lean body mass in trained females. Future Physiology virtual conference, *The Physiological Society*. Interactive poster presentation with virtual small-group discussion of the abstract (April 2021)

1.1.0. Background

Resistance exercise has many health and performance benefits such as improving flexibility, glucose tolerance, stability, body composition, psychological wellbeing, muscular and bone strength, and reducing the risk of age-associated sarcopenia (Anderson & Behm, 2005; Eriksson et al., 1998; Layne & Nelson, 1999; Schoenfeld, 2010; Tan, 1999). For these benefits to be reaped, repeated bouts of resistance exercise are necessary. For instance, the American College of Sports Medicine recommend that resistance exercise be performed 2–4 times per week to optimise muscle strength and hypertrophy adaptations (ACSM, 2009). However, many individuals, especially those who are new to intentional exercise, report difficulty in adhering to exercise training programmes. The reasons for non-adherence are varied, though time commitment, lack of motivation, fatigue, lack of enjoyment, and health problems are commonly cited (Larson et al., 2018; Marzolini et al., 2010; Viken et al., 2019). It is plausible that feelings of fatigue and low motivation to continue exercise are driven by sensations of muscle soreness and stiffness that often arise in the days following an exercise session (Clarkson & Hubal, 2002; Damas et al., 2016$_a$).

Exercise-induced muscle soreness is a consequence of exercise that is unaccustomed (i.e. a previously unperformed movement, intensity, duration, repetition range) or eccentrically-biased (Armstrong, 1984; Ebbeling & Clarkson, 1989; Stauber, 1989). Exercise in which the engaged muscle lengthens during contraction is referred to as 'eccentric' and examples include downhill running, box jumps, and resistance exercise. These forms of exercise can cause acute damage to muscle fibres – a phenomenon that has been termed 'exercise-induced muscle damage' (EIMD). EIMD not only presents symptoms of muscle soreness, but also is associated with various functional impairments. Notably, the capacity for skeletal muscle to generate force can be reduced by ~50% immediately following EIMD, which can take almost a week to restore (Chen et al., 2016; Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002$_b$). Moreover, damage to cell membranes allows the leakage of intramuscular proteins, resulting in supraphysiological concentrations of muscle enzymes within circulation (Nosaka & Clarkson, 1996$_c$). These events, alongside the pro-inflammatory cascade triggered by EIMD (Paulsen et al., 2012), are accompanied by muscle swelling, which can restrict joint range of movement and flexibility (Stauber et al., 1990). Therefore, not only may EIMD lower motivation and

compliance with exercise training programmes, but exercise quality may suffer and training adaptations could be compromised (Damas et al., 2016$_b$).

EIMD is typically magnified in novice exercisers, although experienced exercisers are still impacted by symptoms of EIMD (Ertel et al., 2020; Vincent & Vincent, 1997). Resistance training programmes commonly incorporate progressive overload, whereby the stress placed upon the body is gradually increased by means of manipulating the exercise load, volume, or frequency. Progressive overload is deemed necessary to optimise training adaptations (Kraemer et al., 2002). Therefore, resistance-trained individuals may often perform unaccustomed exercise, and hence are susceptible to frequent muscle damage. Accordingly, several studies have demonstrated that muscle damage, indicated by Z-band streaming, increases during 8–9 weeks of progressive resistance training (Roth et al., 1999; 2000$_a$; Staron et al., 1992). Further, despite being more damaging to muscle fibres than concentric contractions (Margaritelis et al., 2021), eccentric contractions may intentionally be performed to elicit various benefits. Repeated eccentric contractions can induce increases in muscle size and strength (Franchi et al., 2014; Schoenfeld et al., 2017$_c$; Vikne et al., 2006) while comprising a lower metabolic cost than concentric contractions (Lastayo et al., 1999). Moreso, eccentrically-biased exercise may reduce the occurrence of sport injuries (Croisier et al., 2002) and is widely prescribed in injury rehabilitation programmes (Croisier et al., 2007; Hody et al., 2019). Therefore, EIMD from eccentric or unaccustomed exercise poses an issue for all exercising individuals – from novices, to amateurs, to elite athletes.

Consequently, the past two decades have seen an increase in research dedicated to understanding the aetiology of EIMD, its mechanisms of action, potential mitigation strategies, and its relevance in the wider context of health and sport and exercise science. Recent developments in the aetiology of EIMD have indicated that the unaccustomed nature of eccentric exercise is a stronger governing factor than the eccentric exercise *per se* (Margaritelis et al., 2021). Nonetheless, it is agreed that muscle damage arising from loaded voluntary contractions (i.e. resistance exercise) is most severe compared to other exercise modalities (e.g. running, cycling, unloaded contractions). Since the establishment of the muscle length-tension relationship in the 1960s (Gordon et al., 1966), many experimental models have examined the influence of numerous resistance exercise variables on the magnitude of EIMD. Accordingly, the intensity, volume, velocity, rest period, and range of motion (ROM) of the exercise may be strategically modified to manipulate the muscle damage response. However, such variables are often poorly isolated in experimental models with humans, and controversy remains as to

which predominates the EIMD response. For instance, many authors claim that high-intensity or maximal contractions induce the greatest myofibre damage, yet examination of the broader evidence does not strongly support this concept. Much like the factors causing or intensifying EIMD, the mechanisms leading to symptom presentation also are misunderstood.

Key discoveries pertaining to the mechanisms of EIMD were made in the 1990s by the groups of Warren and Morgan. These researchers had opposing views on which is the primary event in EIMD – sarcomere damage or excitation-contraction decoupling – a debate that is still unsettled today (Morgan, 1990; Proske & Morgan, 2001_b; Warren et al., 1993). Secondary to these events is the disruption of calcium homeostasis and consequently, the stimulation of degradative autogenic pathways and proinflammatory mediators. The inflammatory response is central to the regeneration of fully functional muscle fibres, occurring around five days following EIMD. However, these mechanisms are only known to underpin the functional and biochemical consequences of EIMD. The development of perceptual symptoms, for instance muscle soreness and pain, are poorly understood. Several mechanisms of muscle soreness have been proposed and dismissed over the past three decades. To date, suggested candidates involve local inflammation or altered connective tissue behaviour (Peñailillo et al., 2015; Philpott et al., 2018), though this research is still in its infancy.

Irrespective of the pathways for its development, the relevance of EIMD is controversial. Some argue that muscle damage and subsequent muscle regeneration are necessary for the occurrence of training adaptations, such as muscle hypertrophy (Evans & Cannon, 1991; Schoenfeld, 2012), hyperplasia, and increases in connective tissue (Nosaka et al., 2003). Others, however, believe EIMD hinders such adaptations (Damas et al., 2016_b; 2018). The likelihood is that both standpoints hold true, as suggested in the theoretical framework presented by Owens and colleagues (2019). Their framework – based on the hormesis theory – depicts a bell-shaped curve by which positive adaptations to the exercise stress occur up to a threshold, beyond which adaptations are impaired when the stress becomes too great. Few studies have directly examined the relationship between EIMD and chronic muscular adaptations; thus, these hypotheses are primarily based on indirect associations. The studies by Roth and colleagues indicate that muscle damage is neither a detriment nor requirement for muscle adaptation. These authors showed that ultrastructural muscle damage is amplified after 9 weeks of progressive resistance training relative to pre-training in older, but not younger, females, yet both age groups experienced strength gains (Roth et al., 2000_a). In males, both younger and older experienced increases in strength and muscle damage during training (Roth et al., 1999).

These studies also highlight that muscle damage is not attenuated with repeated bouts of exercise if training is progressive; although, this is not the case for young females, potentially due to an oestrogen-mediated protection, as discussed later in this thesis. Nonetheless, acute, as opposed to chronic, responses to EIMD are perhaps of greater relevance to exercising individuals, especially competitive athletes. For instance, during periods of heavy fixtures or back-to-back competition whereby muscle recovery is paramount, it may be pragmatic to minimise EIMD and forfeit the adaptative responses. Conversely, EIMD may be intentionally induced throughout light training periods when its consequences (i.e. muscle soreness and impaired muscle function) are less detrimental to athletic success. Therefore, perhaps EIMD must be neither eliminated nor unconstrained, but rather strategically manipulated to support the goals of the individual.

EIMD can be managed through various physiotherapeutic, pharmaceutical, and nutritional means, which could be incorporated before, during, or succeeding an exercise bout. The general consensus supports a place for cryotherapies (Rose et al., 2017), tissue massage (Torres et al., 2012), compression garments (Marqués-Jiménez et al., 2016), and low-intensity exercise (Ma et al., 2020) for attenuating EIMD and/or accelerating muscle recovery. From a nutritional stand-point, antioxidant vitamins have demonstrated little efficacy in the management of EIMD (McGinley et al., 2009), despite their roles in maintaining membrane permeability and protecting against reactive species damage (Frei, 1994; Lucy, 1972). Showing promise, are polyphenol-rich foods (particularly Montmorency cherries, pomegranate, and beetroot), omega-3 polyunsaturated fatty acids, vitamin D, amino acids, and proteins (Harty et al., 2019; Owens et al., 2019). In particular, dietary proteins – either from whole-food or isolated sources – are commonly ingested around the time of exercise, due to their stimulatory effect on muscle repair and growth (Tipton, 2008). It follows that protein would be a convenient nutritional strategy for the management of EIMD; co-benefiting muscle damage and repair processes. While supplemental protein sources, such as powders, gels, and bars are convenient to the consumer, they are typically low in other macro- and micro- nutrients. Many sport nutritionists now promote a ‘food first’ approach, whereby daily nutrient requirements are predominantly met through consuming whole-foods when possible. The food first approach enables several vital nutrients to be obtained from one food-source or meal, and promotes a clean-sport environment, thus supporting multiple health, performance, and moral goals. For instance, milk products (e.g. cow’s milk, yoghurt, cheeses) are a common staple to the Western diet, due to their affordability; accessibility; versatility; and provision of carbohydrates, proteins, fats, and

a range of vitamins and minerals. The nutritional profile of milk products makes them an attractive food to consume peri-exercise, as sufficient intake will stimulate both muscle protein synthesis (MPS) and muscle glycogen resynthesis (Ferguson-Stegall et al., 2011; Wilkinson et al., 2007). By this means, milk products can support exercise adaptations and help maintain overall muscle health (Hartman et al., 2007). However, the effectiveness of milk products in managing EIMD is controversial. Several experimental trials in untrained males have documented a beneficial impact of milk protein ingestion on blood creatine kinase concentration ([CK]), muscle force-generating capacity, and muscle soreness following EIMD compared to control products (Cockburn et al., 2008; Draganidis et al., 2017; Hirose et al., 2013). All the while, others have reported indifferent muscle damage responses to exercise with the ingestion of milk proteins versus carbohydrate (Cockburn et al., 2010; 2012; Rankin et al., 2015; Wojcik et al., 2001). Variation in inter-study methodological design – notably related to the exercise protocols, protein dosing and timing regimes, dietary controls, measurement tools, and participant characteristics – likely contribute to these inconsistent data.

Despite decades of research in this field, there is a major gap in our knowledge of EIMD management due to an underrepresentation of female populations. Although evidence to support sex differences in the EIMD response is ambivalent (Hubal & Clarkson, 2009; Pizza, 2009; Tiidus & Enns, 2009), this does not mean that research should be sex exclusive. Likewise, EIMD management strategies originating from male-dominated research may not be applicable to females, and studies ought to be replicated with inclusive populations. While the need for female-focused research is irrefutable, the ideal research would study males and females synchronously to enable direct comparison and help solidify the role of sex in EIMD.

1.1.1. Research Aims and Questions

The aims of this research thesis are to identify and bridge the sex data gap in EIMD research; understand factors contributing to resistance EIMD; and inform practical recommendations for the management of resistance EIMD. These aims will be addressed by answering the following research questions:

1) What does the current literature suggest about the overall impact of dietary protein on resistance exercise-induced muscle damage? (*chapter 3*)
2) Does the consumption of milk protein impact resistance exercise-induced muscle damage and does this response differ between untrained males and females? (*chapter 4*)

3) Does the total and regional body composition of untrained males and females relate to resistance exercise-induced muscle damage? (*chapter 4*)
4) Is performing low-load resistance exercise an effective strategy for reducing exercise-induced muscle damage? (*chapter 5*)

1.2.0. Methodological Approach

To achieve the aims and objectives of this work, this research implemented a quantitative design to examine EIMD, with a central theme of sex differences. The research comprised both primary data obtained from a randomised controlled trial (RCT) (*chapter 4*) and secondary data obtained through systematic reviewing (*chapter 3*) and scoping (*chapter 5*) of relevant literature. *Figure 1.1* outlines and connects the elements of this thesis, and a detailed methodology is provided in the appendices.

This research adopted a positivist approach with objective epistemological perspectives. The ways of gathering knowledge, and knowledge itself, are subjective. Thereby epistemology describes the generation and transfer of knowledge (Cohen et al., 2007). Closely linked, ontology is concerned with the perception of reality (Crotty, 1998). To this end, two philosophical approaches have been adopted to describe epistemological and ontological perspectives: positivism and interpretivism. From a research perspective, methodological designs are often based on either positivist or interpretivist approaches depending on the nature of the research and the epistemological and ontological stance of the researcher. This section will discuss these approaches and justify the choice of approach for the current research.

Typically, qualitative research adopts an interpretivist approach and is based on the epistemological and ontological perspectives of subjectivism and relativism, respectively (Scotland, 2012). Interpretivism holds the belief that reality is governed by senses and therefore, is determined by an individual's perception and experiences (Guba & Lincoln, 1994). Knowledge gathered from interpretivist research is subjective, fluid, and layered; typically encompassing participants' feelings, emotions, and opinions. On one hand, interpretivist approaches offer flexibility and allow the exploration of complex research questions with wider scope. However, interpretivist research theories are developed after the gathering of data (Cohen et al., 2007), and data are often subjective and context-specific; thus, it is challenging to reach a consensus on the knowledge derived from interpretivist research. Moreover, information provided by the participant may diverge from the information received by the

researcher (Rolfe, 2006), which may be further misconstrued by the reader of the research; as such, knowledge dilution is a limitation of interpretivism.

Opposingly, quantitative research generally adopts a positivist approach, formed from the epistemological perspective of objectivism – based on the gain of factual knowledge – and the ontological perspective of realism, which argues that reality exists independent of individual perceptions. Positivists seek to simplify complex theories and find causes of specified outcomes by obtaining data using methods that are direct, controlled, standardised, and replicable, without a predisposed stance of proving or disproving hypotheses. Data derived from positivist research are objective, verifiable, and seldom influenced by a participant's emotions and personal views. As such, gathered data can be statistically analysed and used to make predictions and generalisations to other contexts. An extension of positivism, post-positivism believes that truth (i.e. knowledge obtained through scientific experimentation) is only one's belief in the truth of the present research hypotheses. Post-positivism approaches deem that truth can never be proven and thus, scientific hypotheses are not accepted, but rather fail to be rejected (Creswell, 2009). A limitation of positivist methodology is the difficulty in controlling all variables and reducing the influence of confounders on the study outcomes. While experimental procedures can be standardised, confounders can be unpredictable and are not always apparent until after the data is gathered (House, 1991). Further, unintentional researcher bias may present itself throughout various stages of research, for example during the selection of outcome measures, measurement tools, sampling strategy, and interpretation of findings. Bias not only may influence outcomes, but sources of bias may also vary upon study replication (Salomon, 1991); hence, mitigation strategies such as randomisation and blinding are practiced.

This thesis adopted positivist and post-positivist approaches, and both primary and secondary data were quantitative in nature. The RCT involved the direct assessment of human physiological responses to exercise, which produced objective, numerical data. Although some subjective measures were included – specifically the assessments of muscle soreness and pressure-pain threshold (PPT) – these produced quantitative data and therefore did not require subjective interpretation by the researcher. The gathered data was used to reject or fail to reject the study's null hypotheses. The systematic and scoping reviews followed a standardised and rigorous procedure to select relevant primary studies that held a positivist approach and produced quantitative data. Data from the systematic review (*chapter 3*) were included in a meta-analysis to objectively determine the impact of protein supplementation on resistance

EIMD. Data from the scoping review (*chapter 5*) were analysed using a semi-quantitative approach to interpret, describe, and collate the study outcomes to reach a consensus on the impact of resistance exercise load on EIMD. Overall, due to the inherently quantitative, controlled, and objective nature of this research, a positivist approach with objective epistemological perspectives was appropriately used to inform the methods.

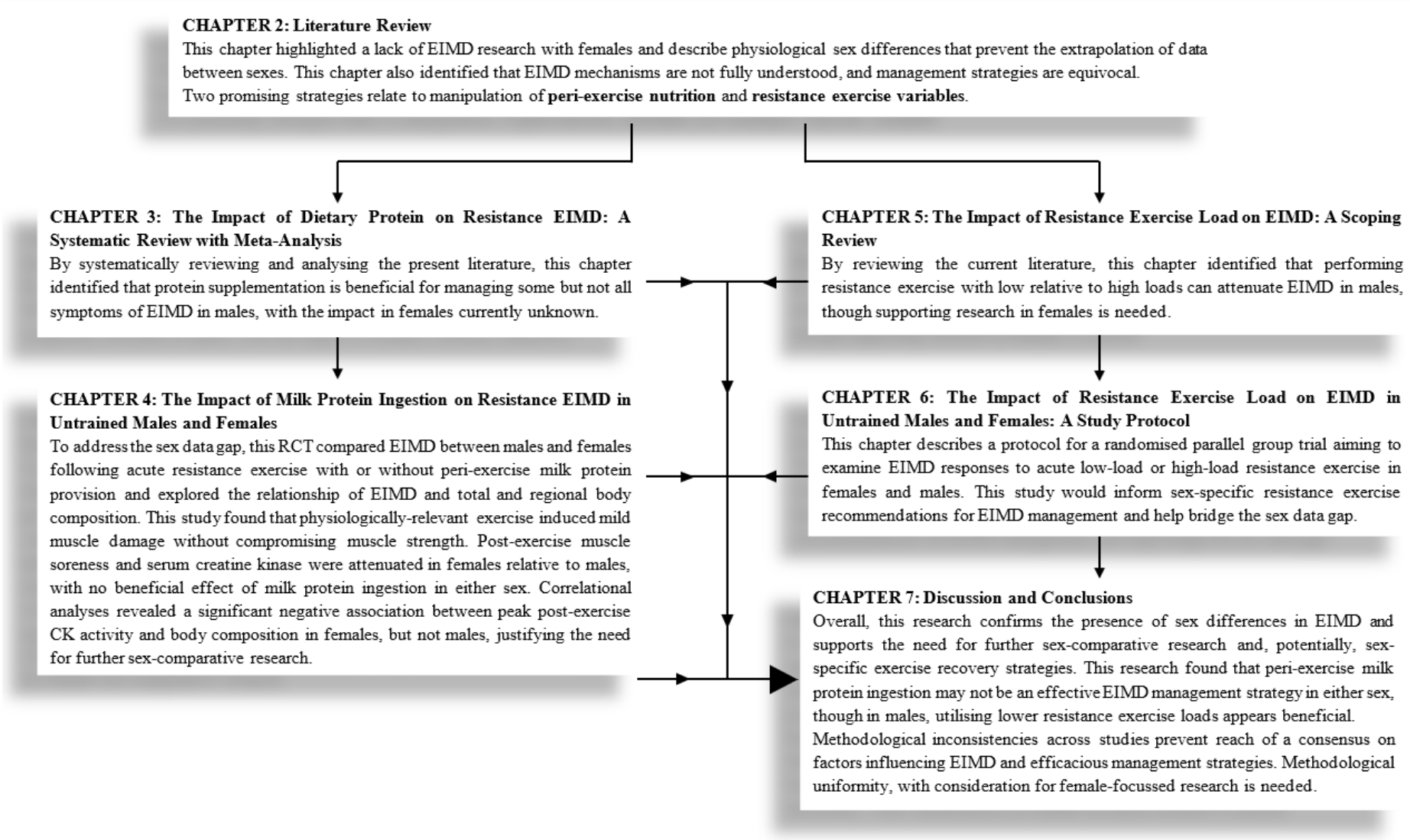


Figure 1.1. Overview of thesis.
EIMD = exercise-induced muscle damage, RCT = randomised controlled trial

2.1.0. Introduction

The underpinning mechanisms of resistance EIMD are discussed in this chapter to provide an understanding of the pathway from muscle contraction-induced tissue damage to symptom development, through to muscle repair and remodelling, with reference to sex differences where appropriate. First, section *2.2.0.* provides an overview of skeletal muscle, including its structure, mechanisms of contraction, fibre types, and factors influencing skeletal musculature. Then section *2.3.0.* focusses on skeletal muscle damage, incorporating the proposed mechanisms of EIMD, experimental markers to quantify EIMD with support from applied data, mechanisms of muscle repair and regeneration, and exercise-induced muscle adaptations.

The current literature also has been explored in this chapter to identify factors impacting EIMD, recognise knowledge gaps, and justify the need to fill such gaps. This work has a central theme of sex differences and where possible, has drawn upon sex comparative research and discussed male and female data separately. Section *2.4.0.* highlights the need for female inclusive research due to the many sex differences in human physiology and responses to nutrition and exercise stimuli. The origin of these differences, being variation in sex steroid hormones both between sexes and within the female lifespan, is discussed with reference to methodological considerations for conducting research with female participants. Considering chapters 3 and 4 concentrate on the role of dietary protein as a management strategy for EIMD, section *2.5.0.* of this chapter introduces protein and outlines skeletal muscle protein metabolism and current dietary intake recommendations for protein dose, timing, and type. Finally, resistance exercise is briefly covered in section *2.6.0.*, with emphasis on the extrinsic and intrinsic variables that can influence acute and chronic resistance exercise-induced muscle adaptations.

2.2.0. Skeletal Muscle

Skeletal muscle is a highly plastic tissue that comprises approximately 40% of an individual's total body mass. Skeletal muscle is predominantly composed of water and protein, with up to 75% of total body proteins being contained within muscle. Accordingly, skeletal muscle accounts for a high proportion (30–50%) of whole-body protein turnover and undergoes constant remodelling. By this means, the mass of skeletal muscle is determined by the rate of protein turnover (see section *2.5.1.*). The functions of skeletal muscle may be defined as being mechanical or metabolic in nature. From a mechanical perspective, skeletal muscle enables movement, generates force, and maintains posture to support daily activities and overall health. Metabolically, skeletal muscle is involved in the storage and metabolism of substrates, basal energy metabolism, thermoregulation, and protein synthesis. Therefore, the maintenance of skeletal muscle mass and function is imperative for overall health and wellbeing. An anatomical depiction of skeletal muscle is presented in *Figure 2.1.a*.

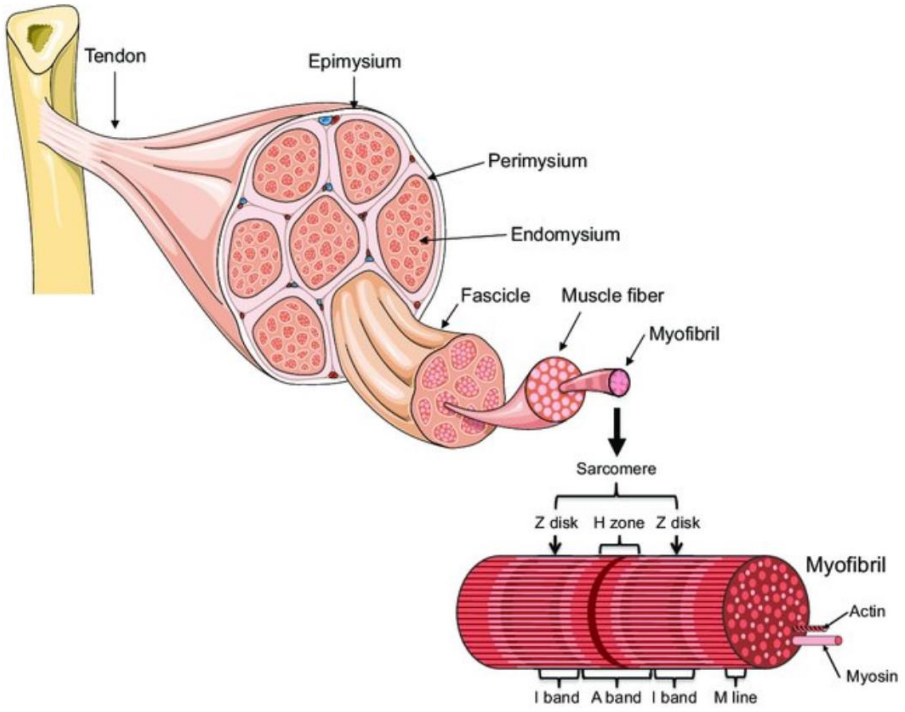


Figure 2.1.a. Anatomical structure of skeletal muscle (top) and a skeletal myofibre (bottom). Image from Bone and Muscle, Basic and Applied Bone Biology, pp. 319 (Bonetto & Bonewald, 2019).

2.2.1. Mechanisms of Skeletal Muscle Contraction

Skeletal muscle contractions are either isometric (muscle length is unchanged) or isotonic (tension may or may not change while the muscle length changes). During an isotonic

contraction, the muscle length shortens (concentric contraction) or lengthens (eccentric contraction) (Hill, 1925). The mechanics of muscle contraction remain in line with the original 'sliding filament' theory proposed in 1954 (Huxley & Hanson, 1954). Briefly, muscle contractions are dependent on the availability of adenosine triphosphate (ATP). The binding action of ATP to myosin causes the dissociation of myosin from actin and the hydrolysis of ATP to release a phosphate molecule, which remains bound to the disengaged myosin. The myosin head then forms a new cross-bridge with the adjacent actin molecule, followed by the release of phosphate, which triggers a force-generating 'power stroke' that causes the actin filament to be pulled toward the M-line in a sliding motion. This process is repeated, as the muscle is stretched under tension until actin and myosin can no longer form cross-bridges and muscle force is lost (Frontera & Ochala, 2015).

Muscle contraction is initiated when a signal from the central nervous system reaches the axon terminal at the neuromuscular junction of the muscle fibre. Subsequently, acetylcholine is released from synaptic vesicles within the axon terminal, travels across the synaptic cleft, and binds to its receptors located on the sarcolemma, causing the opening of membrane ion channels and the exchange of Na^+ and K^+ in and out of the muscle fibre, respectively. The influx of Na^+ depolarises the membrane, and once a particular threshold voltage is reached, an action potential is generated and stimulates excitation-contraction (E-C) coupling (Kuo & Ehrlich, 2015). Membrane depolarisation also stimulates Ca^{2+} release from the adjacent sarcoplasmic reticulum and a steady influx of Ca^{2+} into the cytoplasm is mediated by ryanodine receptor (RyR) Ca^{2+} channels (Field et al., 1988; Lamb & Walsh, 1987). Ca^{2+} release immediately stops upon the deactivation of RyR channels, and any remaining cytoplasmic Ca^{2+} is pumped back into the sarcoplasmic reticulum (Lamb, 2000).

Calcium ions enable muscle contraction by binding to troponin – a regulatory protein on the actin filament. In the absence of Ca^{2+}, the binding site of troponin would be occupied by tropomyosin, which inhibits the interaction between actin and myosin and, by this means, muscle contraction (Gomes et al., 2002). During a series of muscle contractions, $[Ca^{2+}]$ drops, and tropomyosin reoccupies the binding site on troponin; consequently, force production gradually declines and muscle fibres eventually fatigue (Allen et al., 1995). The initiation and mechanics of muscle contraction are summarised in *Figure 2.1.b*.

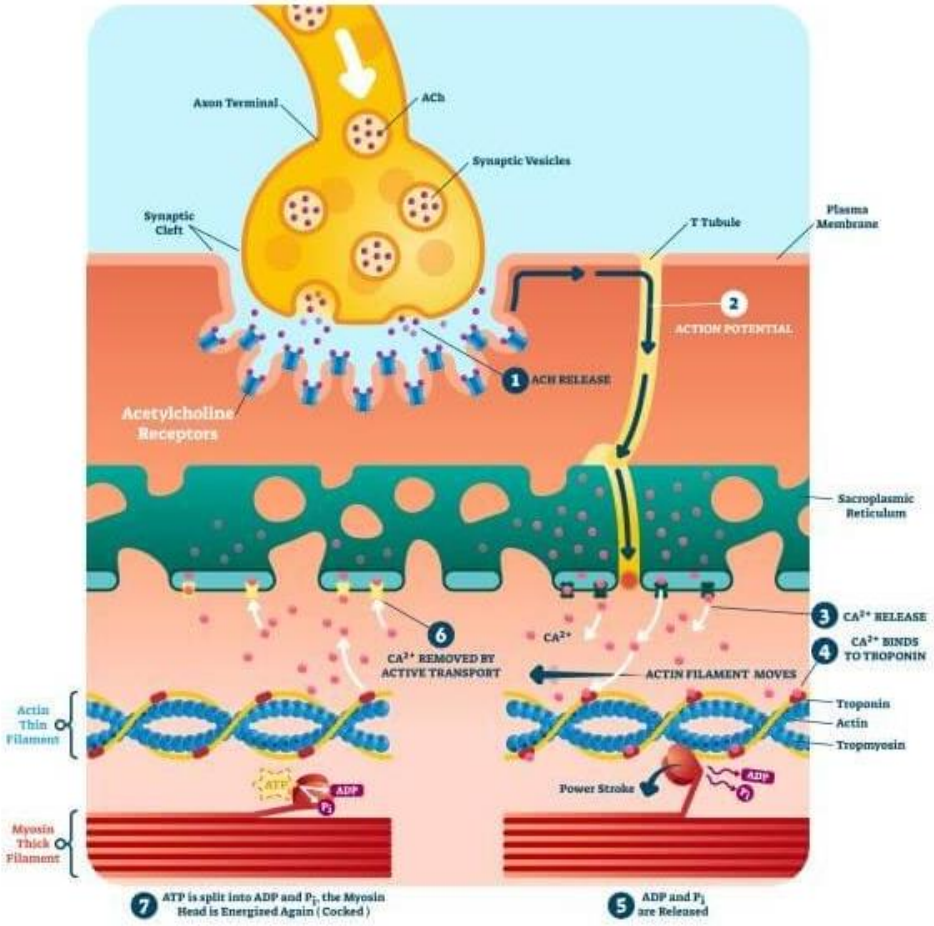


Figure 2.1.b. The initiation and mechanics of skeletal muscle contraction.
Image from: Biologydictionary.net Editors. (2021, February 07). Actin and Myosin. Retrieved from https://biologydictionary.net/actin-and-myosin/

2.2.2. Skeletal Muscle Fibre Types and Sex Differences in Skeletal Musculature

Skeletal muscle fibres may be categorised into three predominant types: type I (slow-twitch), type IIA, and type IIX (fast-twitch), which possess divergent properties. Specifically, type I muscle fibres have a slow contractile speed, house oxidative metabolic pathways, appear red in colour due to their high myoglobin and capillary content, and constitute around 40% of vastus lateralis muscle – the most commonly sampled muscle tissue (Andersen et al., 2000; Scott et al., 2001; Spangenburg & Booth, 2003). Owing to their high fatigue resistance, type I fibres are activated during prolonged aerobic exercise of low intensity (around ≤40% $VO_{2\ max}$) (Vollestad & Blom, 1985). Type IIA fibres comprise 30–35% of vastus lateralis muscle, are red in appearance, and are considered oxidative, though possess intermediate metabolic properties (Scott et al., 2001). Type IIA fibres have a faster contractile speed than type I, are

relatively fatigue resistance and hence, are recruited during moderate intensity endurance exercise (Vollestad & Blom, 1985). Constituting the remaining 25–30% of the vastus lateralis muscle and identifiable by their white colour are type IIX fibres. Although fatigable, with the fastest speed of contraction, type IIX fibres are the dominant fibre type activated during near-maximal aerobic exercise and resistance exercise through the utilisation of glycolytic metabolic pathways (Morton et al., 2019; Vollestad & Blom, 1985). Nonetheless, muscle fibre recruitment operates on a continuum, such that the energy contribution of each fibre type is altered in line with the metabolic stress placed on the muscle.

In accordance with the demands placed upon them, muscle fibres may convert from one type to another and hence are considered plastic (Pette, 2002; Scott et al., 2001). Most common is the adaptive conversion between type IIA and IIX in response to stimuli (e.g. exercise training). For instance, resistance training increases the proportion of type IIA fibres and reduces the amount of type IIX within both male (Campos et al., 2002; Green et al., 1999; Hather et al., 1991; Staron et al., 1994) and female (Staron et al., 1994) skeletal muscle with (Martel et al., 2006) or without (Campos et al., 2002; Green et al., 1999; Hather et al., 1991; Staron et al., 1994) change to type I fibre distribution. This fibre type conversion pattern appears common to healthy adults of both sexes, though whether differences exist in the general composition of skeletal muscle between males and females is debated.

Sexual dimorphism in skeletal musculature exists in a fibre type-dependent manner, with variation in the distribution and size of type I, IIA, and IIX muscle fibres. Sex differentiation in fibre type distribution is apparent during the transition from adolescence to adulthood, when in these developmental years, males encounter a decrease in the proportion of type I fibres (from ~55% to 48%) and an increase in type II fibres (from ~44% to 49%), meanwhile the reverse occurs in females (Glenmark et al., 1992). Once adulthood is reached, similar proportions of type I (36–58% vs. 38–55%), type IIA (27–42% vs. 30–39%), and type IIX (13–22% vs. 12–30%) fibres are typically found within the vastus lateralis of males and females, respectively (Doriguzzi et al., 1984; Essen-Gustavsson & Borges, 1986; Glenmark et al., 1992; Gollnick et al., 1972; Nygaard, 1981; Simoneau & Bouchard, 1989; Simoneau et al., 1985). However, others have measured a greater proportion of type I fibres in females (Glenmark et al., 1992; Simoneau & Bouchard, 1989; Simoneau et al., 1985; Staron et al., 2000), which has been associated with superior fatigue resistance relative to males (Wüst et al., 2008). Conflicting data could be owing to inter-individual variability in fibre type distribution (Staron

et al., 2000), alongside the age, activity level, training status, and hormonal profile of populations studied.

Sex differences in skeletal musculature may pertain to the size, rather than distribution, of muscle fibres. During adulthood, males develop larger muscle fibres (Martel et al., 2006) and superior strength (Bishop et al., 1987) to their female counterparts. Notably, type II fibre hypertrophy is enhanced in males (Glenmark et al., 1992), such that the respective cross-sectional fibre areas of type IIA and IIX are 38–59% and 56–66% greater in males than females (Simoneau & Bouchard, 1989; Staron et al., 2000). Within male muscle, type II fibres are typically larger than type I and occupy a greater total region of muscle. Conversely, in female muscle, type I fibres are superior in size to type II (Brooke & Engel, 1969; Saltin et al., 1977; Simoneau & Bouchard, 1989; Simoneau et al., 1985; Staron et al., 2000). Therefore, it would appear the size of each muscle fibre type and its respective occupation of total muscle are the key characteristics distinguishing male and female musculature.

2.3.0. Muscle Damage

Muscle damage is the excessive stretching of myofibres, resulting in minor tears near the muscle-tendon junction, which present as various physical and subjective symptoms ranging in severity from tolerable to debilitating. Myofibres are overstretched when they are under great tensile force, and therefore, physical exercise is a common cause of muscle damage (Fernandes et al., 2011).

2.3.1. Mechanisms of Exercise-Induced Muscle Damage

Unaccustomed resistance exercise and eccentric muscle contractions, such as those performed during downhill running, are the primary inducers of muscle fibre damage (Allen, 2001; Armstrong, 1984; Ebbeling & Clarkson, 1989; Stauber, 1989). Recently, it has been questioned whether it is the unaccustomed nature of eccentric contractions that drives the muscle damage response, as opposed to the eccentric contraction *per se*. This theory was derived from the observation of a blunted muscle damage response once a muscle is accustomed to eccentric resistance exercise (Margaritelis et al., 2021). However, this notion warrants further investigation. While concentric muscle contractions are capable of causing muscle damage, the consequences are neither as severe nor sustained as those triggered by eccentric contractions (Margaritelis et al., 2021). Eccentric contractions can lead to the weakening or rupture of one

or more structural components of the myofibre – known as 'failing' – resulting in reduced capacity for force generation. Muscle failure may occur if the tensile stress placed on the myofibre component exceeds its yield strength (Armstrong et al., 1991). Eccentric contractions are capable of generating 150–200% of the maximal force of an isometric contraction (Woledge et al., 1985), thereby increasing the vulnerability to muscle failure and muscle damage. McCully and Faulkner (1986) were first to speculate that the greater force-generating capacity of eccentric contractions is not the only characteristic involved in muscle damage. It was demonstrated by these authors that eccentric contractions generating 85% of maximal isometric force induced muscle damage, but concentric contractions at the same intensity did not (McCully & Faulkner, 1985). Therefore, the muscle length at which maximum force is produced, rather than the force itself, may determine the degree of damage. Peak force during an eccentric contraction occurs when the muscle is stretched to 110% of its resting length, relative to 100% of resting muscle length during concentric contractions (McCully & Faulkner, 1985). In agreement, a positive relationship between the length at which eccentric contractions were initiated and the severity of muscle injury incurred was demonstrated by Newham and colleagues (1988). As sarcomeres increase in length, there is a reduction in actin-myosin overlap, of which the consequences are two-fold. First, there is loss of tension as the number of myosin heads engaged with the actin filament decreases and second, there is an increase in energy dissipated as heat or used in the deformation of the sarcolemma or its components (Stauber, 1989; Tidball & Daniel, 1986). Accordingly, the greater lengthening capacity and force production of eccentric contractions might explain why they induce more severe muscle damage than concentric contractions (Margaritelis et al., 2021). The various proposed mechanisms of eccentric contraction-induced muscle damage and its physiological effects are now discussed. Of note, this early mechanistic research has predominantly been conducted using animal or *in vitro* models; however, later studies with humans that have induced and examined muscle damage do not indicate alternative mechanisms.

Excitation-Contraction Coupling Dysfunction

There are two prominent features of muscle damage which occur immediately after repeated eccentric contractions, being disruption to the E-C coupling system and damage to sarcomeres. Is it debated which is the primary event i.e. whether sarcomere damage causes E-C coupling dysfunction or *vice versa* (Proske & Morgan, 2001b; Warren et al., 1993). Disruption to the E-C coupling system was argued by Warren and colleagues to be the primary event in muscle damage following eccentric contractions. Their hypothesis was supported by the exposure of

mouse soleus muscle to caffeine after a series of damaging eccentric or non-damaging isometric contractions. Caffeine directly activates the RyR channels and allows Ca^{2+} influx (Martonosi, 1984) so, if muscle damage were to be attenuated with caffeine exposure, it would suggest that insufficient Ca^{2+} release, and thereby failure of E-C coupling, was the cause of muscle damage. Damaged muscle exposed to caffeine was demonstrated by Warren et al. (1993) to produce comparable maximal force to non-damaged muscle, thus placing E-C coupling dysfunction as the primary event. In support, an *in vitro* approach is to expose muscle fibres to a Ca^{2+} ionophore (ion transporter), which increases Ca^{2+} transport across the sarcolemma and stimulates Ca^{2+} release from the sarcoplasmic reticulum. Elevated free cytosolic [Ca^{2+}] (Goodman, 1987; Publicover et al., 1978) rapidly deteriorates myofibrillar structure and induces the loss of intramuscular enzymes into the medium (Duncan & Jackson, 1987). These events occur in a dose-dependent manner, such that when the ionophore concentration is low (low cytosolic [Ca^{2+}]), protein degradation occurs in the absence of change to myofibrillar structure or maximum tension in rat soleus muscle (Kameyama & Etlinger, 1979). Failure of the E-C coupling system appears to be resolved after 14 days following muscle damage, though in the first five days may account for up to 75% of the reduction in force generation (Ingalls et al., 1998). However, in other species, including amphibians, potentiating Ca^{2+} release does not rescue the fall in muscle tension, indicating that E-C coupling failure is not always the primary event in muscle damage (Allen, 2001; Morgan et al., 1996).

Sarcomere Disruption

Muscle damage may be initiated by damage to sarcomeres. Sarcomeres extend during eccentric contractions, alongside increased muscle tension. However, once a sarcomere exceeds its threshold length, muscle tension will rapidly fall as fewer myosin heads can form cross-bridges with actin – so called the 'length-tension relationship' (Gordon et al., 1966). It has been proposed that the overextension of sarcomeres occurs in the weakest first, then in the second weakest and so on, resulting in a random distribution of overextended sarcomeres within the myofibril (Morgan, 1990). Sarcomeres are disrupted if they fail to re-interdigitate upon relaxation (Talbot & Morgan, 1996) and thus, repeated eccentric contractions can result in the cumulative overextension and disruption of sarcomeres to the point where membrane damage occurs (Morgan, 1990). First, by the tearing of transverse tubules and second, by damage to the sarcoplasmic reticulum (Proske & Morgan, 2001b), ultimately impairing the E-C coupling system. Consequently, free cytosolic Ca^{2+} levels are elevated due to both increased membrane permeability and reduced Ca^{2+} sequestering by the sarcoplasmic reticulum (Byrd et al., 1989_a;

1989$_b$). Mitochondria can act as Ca^{2+} buffers and uptake some of the excess Ca^{2+}; however, once their stores are at maximum capacity, Ca^{2+} will rapidly spill back into the intracellular space (Gissel, 2005). It is agreed that elevated cytosolic [Ca^{2+}] is central to the muscle damage response (Baracos, 1984; Jackson et al., 1984).

Elevated Cytosolic Calcium Concentration

The importance of maintaining Ca^{2+} homeostasis is evidenced by the presence of at least seven systems involved in Ca^{2+} transportation across the sarcolemma (Carafoli, 1985). Disruption to Ca^{2+} homeostasis can trigger a cascade of autogenic responses, including local inflammation, the activation of proteolytic pathways, and increased production of reactive oxygen and nitrogen species (ROS and RNS). Importantly, degeneration processes are restricted to the local site of injury and do not affect the entire myofibre (Armstrong et al., 1983; Carpenter & Karpati, 1989; Ogilvie et al., 1988). Therefore, uncontrolled Ca^{2+} influx occurs only locally and if the cell can maintain a relatively low cytosolic [Ca^{2+}], the autogenic response may not be initiated. Conversely, if several regions of the sarcolemma are damaged and cytosolic [Ca^{2+}] is highly elevated, the activation of autogenic pathways triggers irreversible muscle damage and ultimately cell death (Armstrong et al., 1991; Duchen, 2004). However, the subsequent inflammatory cascade triggered by cell necrosis is vital to muscle regeneration, as discussed in section *2.3.3.*

Elevated [Ca^{2+}] induces muscle damage in three key stages. First, particular cell structures are degraded by Ca^{2+}-dependent proteases (mainly calpain) resulting in Z-band streaming, A-band disruption (Friden et al., 1983; Ogilvie et al., 1988), and fragmentation of titin (Raynaud et al., 2005; Sorimachi et al., 1996). In human studies, recreationally active males (Cermak et al., 2012; Damas et al., 2016$_b$; Macaluso et al., 2014; Shepstone et al., 2005; Yu et al., 2004) and premenopausal (Roth et al., 2000$_a$) and postmenopausal (Singh et al., 1999) females performing high-intensity eccentric, concentric, or plyometric exercise have demonstrated significant Z-band streaming and titin fragmentation, which has been associated with elevated post-exercise calpain-3 activity (Macaluso et al., 2014; Murphy et al., 2007). Peri-exercise consumption of calcium channel blockers may attenuate structural damage to sarcomeres (Beaton et al., 2002), but inhibiting Ca^{2+}-activated proteases does not always reduce damage (Rodemann & Goldberg, 1982). This indicates that either supraphysiological intracellular [Ca^{2+}] overwhelms the effects of protease inhibitors, or that Ca^{2+}-activated proteases are not the main driver of muscle degradation.

Second, high levels of intracellular Ca^{2+} may activate the Phospholipase A_2 (PLA_2) pathway. The PLA_2 enzyme is responsible for the production of arachidonic acid and, by extension, prostaglandins, leukotrienes, and thromboxanes, which mediate pain and inflammation (Korotkova & Lundberg, 2014) and can further degrade phospholipid membranes (Jackson et al., 1984). *In vitro* studies have demonstrated reduced loss of intramuscular enzymes with the addition of PLA_2 or prostaglandin inhibitors to a high [Ca^{2+}] medium (Jackson et al., 1984; Rodemann & Goldberg, 1982), which highlights the role of the PLA_2 pathway in membrane degradation. In studies with humans, elevated plasma levels of arachidonic acid and thromboxane have been recorded after 60 min of cycling exercise in recreationally active males (Laustiola et al., 1984), while marathon running triggered a rise in plasma prostaglandin concentration in males and females (Demers et al., 1981). Conversely, plasma prostaglandins were not elevated following eccentric elbow flexions in untrained males (Hirose et al., 2004) and females (Conceição et al., 2012). Activation of the PLA_2 pathway has been related to both hormonal status (lower response in premenopausal HC users than postmenopausal females) (Conceição et al., 2012) and resistance exercise intensity (Uchida et al., 2009); thus, disagreements might be due to varied exercise protocols and participant characteristics.

Third, mitochondrial Ca^{2+} overload induces oxidative stress via increased ROS and RNS production, leading to peroxidation of membrane lipids and reduced structural integrity of cells (Grijalba et al., 1999). Post-exercise oxidative damage has been documented in recreationally active and well-trained males following downhill running (Close et al., 2004; 2005), exhaustive running (Watson et al., 2005), cycling (Ashton et al., 1998; Koska et al., 2000), and leg-based resistance exercise (Bailey et al., 2007; Bloomer et al., 2005). The specificity of oxidative damage was demonstrated by Bloomer and colleagues (2005; 2006) who reported exercise-induced peroxidation of proteins, but not lipids, which suggests that proteins are targeted first and perhaps that more severe damage is required for lipids to be affected. Accordingly, significant lipid peroxidation did not occur in recreationally active females or well-trained female rowers or footballers following exhaustive running (Mazani et al., 2018), downhill running (Sacheck et al., 2000), or 90 min match-play (Andersson et al., 2010), respectively. Conversely, eccentric resistance exercise triggered significant oxidative damage in untrained females (Goldfarb et al., 2005; Radák et al., 1999), which alludes to an influence of training status and/or exercise type on oxidative species production. Further research is needed to understand the factors impacting exercise-induced oxidative stress, as well as the other Ca^{2+} mediated consequences of exercise (e.g. degradation of cell structures, activation of PLA_2

pathway). In particular, the role of sex is poorly understood due to a lack of research conducted with both males and females.

To summarise, EIMD is initiated by local disruption to sarcomeres and dysregulation of the E-C coupling system. Following these events, Ca^{2+} homeostasis is lost, resulting in critically elevated cytosolic Ca^{2+} concentrations and the activation of autogenic pathways. Subsequently, there is an oxidative stress response, stimulation of the PLA_2 pathway, and activation of proteases. These events damage the structural components of sarcomeres and their phospholipid membranes, ultimately triggering cell death and an inflammatory cascade that is imperative for effective tissue repair and regeneration. Since much of the early research has been conducted with rodents and, later, in humans of one sex (predominantly male), it is not yet possible to characterise sex differences in the mechanisms of EIMD. The process of EIMD is summarised in *Figure 2.2.*

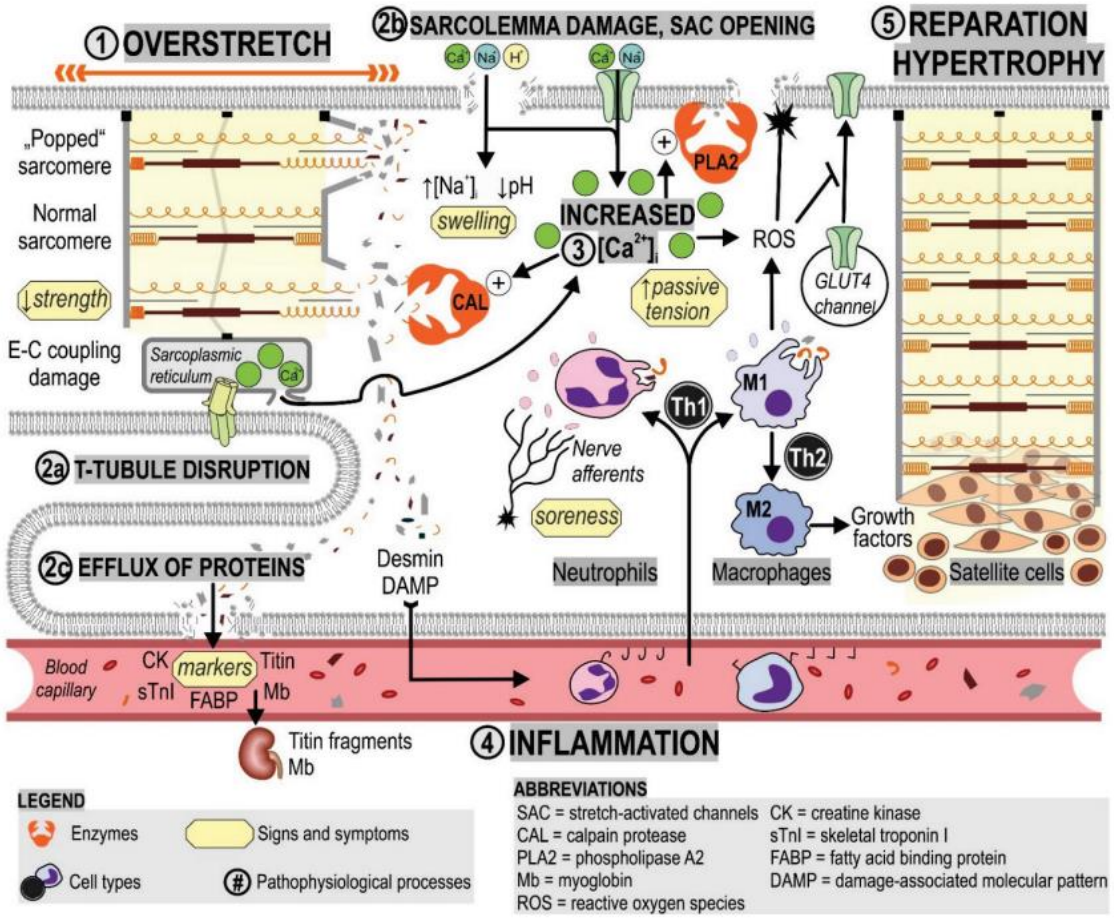


Figure 2.2. Summary of the process of exercise-induced muscle damage. Figure from Stožer et al. (2020) pp. 567.

2.3.2. Markers of Muscle Damage

Several markers may be used to indicate muscle damage in males and females, which can be categorised into direct and indirect markers. Measuring direct markers of muscle damage is challenging as it necessitates either magnetic resonance imaging (MRI), which may not be feasible due to financial, time, and resource constraints, or the collection of muscle biopsy samples. The analysis of muscle biopsy samples using histology, immunohistochemistry, or electron microscopy (Walters & Baborie, 2020) may reveal structural disturbances to the myofibril, including Z-line streaming, loss of myosin filaments, loss of mitochondria, and altered arrangement of A-band filaments (Friden et al., 1983). Nevertheless, there are two inherent assumptions that require consideration when assessing muscle damage from biopsy samples. First, that the degree of damage within the sample reflects the degree of damage within the whole muscle. Second, that the muscle damage is induced by the experimental intervention and not the biopsy procedure itself (Clarkson & Hubal, 2002; Malm et al., 2000). Due to these limitations and the invasive nature of muscle biopsies, indirect markers are more commonly used to assess muscle damage. However, indirect markers can only provide a proxy measure of the magnitude of damage that has occurred and cannot objectively quantify tissue damage, e.g. the number of sarcomeres that have been damaged. Therefore, obtaining several indirect markers, particularly those which may be related (e.g. muscle swelling and inflammatory biomarkers) is beneficial to create a more accurate picture of the magnitude of muscle damage. Ratings of muscle soreness, blood biomarker analysis (including intramuscular and inflammatory proteins), and maximal strength assessments were reported by Warren and colleagues (1999) as being the most frequently assessed indirect measures of muscle damage. *Figure 2.3* shows a time-course of the peak change of these indirect muscle damage markers.

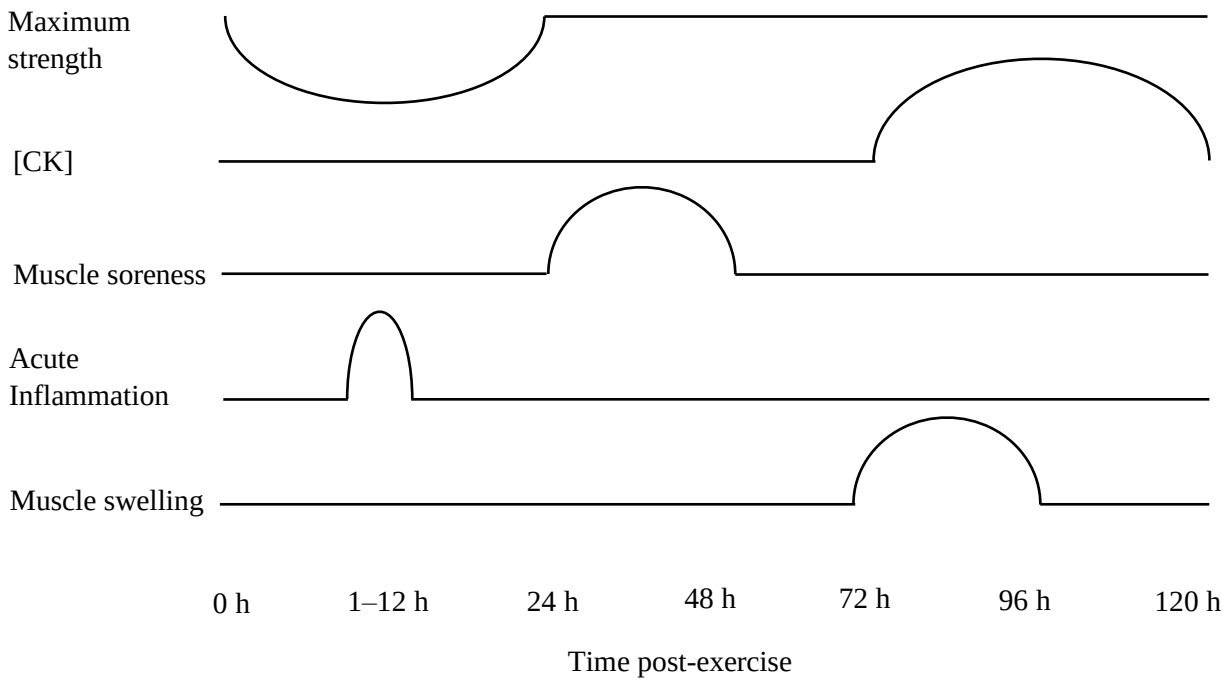


Figure 2.3. Time-course of peak changes in indirect markers of muscle damage. Figure drawn using data from Clarkson and Hubal (2002); Damas et al. (2016).

Muscle Function

Various measures of muscle function including limb flexibility (Chen et al., 2020; Kim et al., 2017), strength, countermovement jump (Orssatto et al., 2018), speed (Rankin et al., 2015), agility (Cockburn et al., 2013), and sport-specific exercise performance (Draganidis et al., 2013; Philpott et al., 2018) are used to indicate muscle damage. The post-exercise change in maximal voluntary contraction (MVC) is deemed to best reflect the magnitude of muscle damage (Damas et al., 2016$_a$). Typically, isometric (Bartolomei et al., 2017; Buckley et al., 2010; Chen et al., 2020; Dale et al., 2015; Draganidis et al., 2017; Nieman et al., 2020; Orssatto et al., 2018; West et al., 2017) or isokinetic (Cockburn et al., 2010; 2012; Cooke et al., 2010; Gee et al., 2019; Grubic et al., 2019; Ives et al., 2017; Saracino et al., 2020) muscle contractions are performed during MVC assessments with use of an isokinetic dynamometer. Less frequently used to determine MVC are one-repetition maximum (1RM) tests (Barroso et al., 2011; Draganidis et al., 2013; Naclerio et al., 2020) or eccentric-only muscle contractions (Paschalis et al., 2005). Different types of muscle strength could be affected by EIMD in varied ways. For example, post-exercise reductions in eccentric and isometric peak torque of ~30% and ~15%, respectively, were documented by Paschalis and colleagues (2005) in untrained

males. This difference may be because the muscle damage exercise protocol was eccentrically-biased, although this idea has been contested elsewhere (Margaritelis et al., 2021). As such, it may not be viable to compare MVC change data between studies that utilised varied methods for its assessment.

Concentric muscle contractions can induce strength loss of up to 36% shortly after exercise in males and females (Cadore et al., 2018; Willoughby et al., 2003), which has been mainly attributed to neural and metabolic fatigue (Beck et al., 2012; Edwards et al., 1977) with no evidence of ultrastructural damage (Newham et al., 1983). Although, sustained (5 days post-exercise) decrements in maximal strength have been documented in young males following concentric contractions (Margaritelis et al., 2021), which were unlikely driven by acute neural responses. The most prolonged and marked reductions in strength occur after high-load eccentric contractions, which can diminish muscle force-generating capacity by ~40–55% that usually takes >5 days to recover to pre-exercise levels (Chen et al., 2016; Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002$_b$). However, when utilising maximal strength assessments for the interpretation of muscle damage there are several methodological limitations that are often ignored in experimental studies involving humans:

1) *Maximal strength tests may cause additional muscle damage*. Assessments of maximal strength are often conducted immediately or shortly after EIMD at the nadir of muscle strength. It is plausible that maximal strength tests, particularly those involving eccentric contractions, induce some degree of muscle damage by placing additional strain on the already disrupted myofibres (Arazi & Asadi, 2013). Therefore, subsequent strength assessments, i.e. at 24, 48, 72+ h after the original exercise bout may cause compounded strength reductions and overestimate the time required for strength recovery. As such, to reflect real-life training practices whereby a muscle group would be rested for day or two after the initial exercise bout, it is perhaps pragmatic to conduct maximal strength assessments ≥48–72 h following EIMD.

2) *Repeated bout effects from pre-exercise maximal strength assessments.* The repeated bout effect (RBE) is an established phenomenon whereby completion of prior exercise attenuates the muscle damage response to subsequent exercise of a similar nature (Hough, 1902; Nosaka & Aoki, 2011) (see section *2.3.4.*). Hence if the protocol used to assess maximal strength shares characteristics with the exercise protocol later used to induce muscle damage, then EIMD may be attenuated if a sufficient washout period is not allocated. For instance, knee extensor eccentric peak torque was assessed by Paschalis and colleagues (2005) prior to a muscle

damaging exercise protocol involving eccentric knee extensions, which might have been exposed to RBEs. Considering RBEs can be evident for up to 9 months following the initial exposure to the contractile stimuli (Nosaka et al., 2001_a), it may be challenging to obliterate the effects completely. Extended periods (>9 months) between baseline and subsequent measures with abstinence from strenuous activity would be required, which is unrealistic for experimental studies with human participants. The magnitude of the RBE is reduced as the time between exercise bouts extends (Nosaka et al., 2005). Accordingly, investigations of EIMD could 1) employ different protocols for assessing strength and inducing damage (with the former being less damaging than the latter) and 2) extend the washout between these two protocols as much as is feasible.

3) *Repeated-bout effects in crossover study designs.* Intervention trials with two or more arms that use a crossover design often have an insufficient washout period between conditions to mitigate any RBEs. Although some washout periods exceed 6 weeks (Draganidis et al., 2017), others as short as 7–10 days have been practiced (Bartolomei et al., 2017; West et al., 2017) and therefore, muscle damage will likely be attenuated during succeeding exercise bouts relative to the first. Further, not all studies counterbalanced group allocation to manage order effects (Paschalis et al., 2005; Peake et al., 2006), which might have intensified RBEs. The influence of RBEs can be negated by using a contralateral limb model, i.e. one limb performs one condition, and the contralateral limb performs the alternative condition. Although, outcomes may be affected by limb dominance, and a washout period is still required.

4) *Failure of untrained individuals to achieve true maximal strength.* Individuals who are unaccustomed to resistance exercise are unlikely to reach their true maximum strength on their first attempt (Babault et al., 2001), especially if the assessment is conducted using free-weights rather than loaded machines or isokinetic dynamometry. It is therefore recommended that baseline strength assessments are conducted twice in novice individuals; first, to estimate and second, to confirm maximal strength. Alternatively, when assessing dynamic strength, multiple-repetition maximum (e.g. 3RM, 10RM) tests can be conducted rather than 1RM tests (ACSM, 2009). However, with some exceptions (Barroso et al., 2011), this guidance is rarely followed in experimental models of EIMD. Novice individuals may more likely achieve their true maximal strength during post-EIMD strength assessments once some neural adaptations to the contractile stimuli have occurred (see section *2.6.2.*). So, underestimation of baseline maximum strength may then underestimate the exercise-induced change in maximum strength. In agreement, 35% of untrained participants were excluded from data analysis by Buckley et

al. (2010) as they failed to demonstrate reductions in isometric force following a damaging exercise protocol. Here, strength preservation was attributed to a failure of the novice individuals in achieving their true maximum strength at baseline. Since this issue is less apparent in those experienced in resistance exercise, it may not be appropriate to compare or combine maximal strength change data collected from trained and untrained individuals.

Blood Markers

A characteristic of EIMD is an increase in cell membrane permeability (see section *2.3.1.*). As a result, intramuscular proteins, such as CK, myoglobin, troponin, and lactate dehydrogenase, may leak into systemic circulation and measuring their respective concentrations within plasma or serum indicates the magnitude of EIMD. In particular, [CK] is frequently measured for the assessment of EIMD. Elevated circulating [CK] can be measured within 24 h after resistance exercise (Nosaka et al., 2002$_b$; Paschalis et al., 2005), but peak values (~2,000–10,000 IU) are not reached until 3–7 days post-exercise (Clarkson & Hubal, 2002) and may take up to 10 days to return to pre-exercise levels (Manfredi et al., 1991). However, the blood concentration of CK and other intramuscular proteins is not only the product of its release from the muscle, but also its clearance from the blood. Various factors including but not limited to, baseline [CK] (Nosaka & Clarkson, 1994), subsequent exercise (Saxton & Donnelly, 1995), number of CK autoantibodies, and sex (Warren et al., 2006) can influence the rate of blood CK clearance. Consequently, highly variable [CK] at rest and in response to exercise can be expected (Nosaka & Clarkson, 1996$_c$) and thus CK data should be interpreted with caution and ideally alongside other markers when used to judge the magnitude of EIMD.

Pro-inflammatory biomarkers also are used in the assessment of EIMD to indicate local and systemic inflammatory responses. It has been debated whether exercise-induced inflammation is a “good” or “bad” process considering its links to muscle pain, soreness, and swelling, as well as to muscle regeneration and repair (Malm, 2001; Tidball, 2011; Toumi & Best, 2003). The post-exercise inflammatory response, although not always present (Kanda et al., 2014; Nosaka & Clarkson, 1996$_b$; Uchida et al., 2009), is marked by a leukocyte-mediated infiltration of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α and various interleukins (IL-1β, IL-6, IL-8) that may be detected within plasma 0–4 h following exercise (Paulsen et al., 2012). For instance, 2 h of treadmill running immediately elevated plasma concentrations of TNF-α and several ILs in endurance trained males and females (Konrad et al., 2011). Likewise, a significant rise in plasma IL-6 was recorded 0–4 h after downhill running

in untrained males and females (Zhao, et al., 2020), but not when the same exercise protocol was implemented in females alone (Koenig et al., 2016), suggesting a sex-specific protection against inflammation, which has been supported elsewhere (Aragón-Vela et al., 2021; Benini et al., 2015). Conversely, a sex-dependent response to whole-body resistance exercise was refuted by Heavens et al. (2014) since both trained males and females experienced a ~600% increase in serum IL-6. Conversely, trained females who performed 300 maximal eccentric leg extensions experienced increases in serum IL-8, IL-10, and TNF-α, but not in IL-6 or IL-1β, suggesting that different exercise stimuli may trigger varied cytokine responses. Accordingly, the resistance exercise-induced infiltration of IL-6 has been linked to exercise intensity (Bartolomei et al., 2017; Mendham et al., 2011; Peake et al., 2006) and contraction type (Willoughby et al., 2003) in untrained males, although overall, systemic inflammation is often unrelated to the severity of EIMD (Buckley et al., 2010; Kanda et al., 2013; Nosaka & Clarkson, 1996$_b$; Peake et al., 2006). It has been proposed that the plasma increase in IL-6 is majorly attributed to intramuscular IL-6 production as a direct consequence of muscle contraction, in the absence of muscle damage *per se* (Steensberg et al., 2000). Accordingly, muscle biopsy samples have revealed increased messenger ribonucleic acid (mRNA) expression of IL-6, IL-1β, IL-8, and TNF-α during the 24-h period following resistance exercise (Paulsen et al., 2012), although it should be noted that the biopsy procedure in itself may induce inflammation (Malm et al., 2000; Van Thienen et al., 2014). Nonetheless, there is emerging agreement that this initial inflammatory response is central to muscle regeneration (Tidball et al., 2014) (section *2.3.3.*), as marked by a shift in pro-inflammatory agents to anti-inflammatory (Chazaud, 2016; Tidball et al., 2014).

Muscle Swelling

The exercise-induced inflammatory response causes oedema, which presents as muscle swelling that typically peaks 4–5 days after exercise (Damas et al., 2016$_a$). Measures of muscle swelling may be used to suggest inflammation in replacement of, or in addition to, blood or biopsy sampling. Techniques, such as MRI, can detect various signs of oedema/swelling, including inflammation of muscle fibres, expansion of the extracellular matrix, augmented muscle volume, alterations in fibre shape, and changes in transverse relaxation time (T2) (Clarkson & Hubal, 2002; Kalia et al., 2017). However, a more convenient and indirect method to examine muscle swelling is the measurement of limb circumference with standard anthropometric techniques. There is evidence that limb circumference expands in an exercise intensity-dependent manner (Chen et al., 2020; Hasenoehrl et al., 2017; Nosaka & Newton,

2002; Nosaka et al., 2002$_b$), although increases in upper arm circumference have been recorded in untrained males following repeated eccentric elbow flexions performed at as low as 10% of maximal exercise intensity (Chen et al., 2020; Peake et al., 2006). Conversely, upper arm circumference remained unchanged in untrained males during the 96-h period following submaximal and maximal eccentric elbow flexor exercise, despite significant muscle damage (Barroso et al., 2011). Findings in females are equivocal; the series of experiments by Brown and colleagues reported that the completion of 15 × 30-m sprints by team-sport athletes and recreational dancers was sometimes (Brown et al., 2015) but mostly not (Brown et al., 2018; 2019; Keane et al., 2015) associated with increased calf and thigh girth. Granted that biomarkers of inflammation were not analysed by these authors, the lack of exercise-induced muscle swelling could be down to a female-driven protection against inflammation (Aragón-Vela et al., 2021; Benini et al., 2015; Tiidus, 2003), although few studies have directly compared muscle swelling in females relative to males. Comparable increases in arm circumference in males and females following eccentric elbow flexions were reported by Dannecker et al. (2012), though further studies are required to corroborate these findings. Moreover, despite limb circumference measures of muscle swelling being convenient, time-efficient, and requiring minimal resources, outcomes are not always related to biomarkers of inflammation (Peake et al., 2006) and therefore, additional measures of inflammation (i.e. blood sampling, biopsies, or MRI) should be obtained if possible.

Range of Motion

The ROM of the exercised limb may be limited following EIMD as a consequence of muscle swelling. Swollen tissue applies pressure to the muscle fascia and passively shortens the resting length of the muscle (Stauber et al., 1990). Individuals may therefore experience sensations of muscle stiffness and reduced flexibility, which could hinder exercise performance. Accordingly, muscle stiffness and ROM have been related to strength loss (Chen et al., 2011; McHugh et al., 1999), and increasing muscle flexibility prior to exercise can (Chen et al., 2011; LaRoche & Connolly, 2006), but not always (Brusco et al., 2018; Eston et al., 2007), reduce symptoms of EIMD. Therefore, assessing joint ROM or muscle flexibility is a useful indicator of the severity of and recovery from EIMD.

Joint ROM is typically measured as the difference between the flexed angle and relaxed angle of the limb joint during a passive contraction using a goniometer, while the sit-and-reach test is commonly used to assess hamstring and back flexibility. Studies have showcased varied

changes in ROM or flexibility of the exercised limb following muscle damage, although peak decreases tend to coincide with peak muscle soreness, i.e. 24–48 h post-exercise. For instance, hamstring flexibility was reduced by ~20% in female recreational dancers 24–48 h after repeated high-intensity sprints (Brown et al., 2018, 2019), whereas hamstring flexibility was unaffected by 60 min of downhill running in female runners (Köhne et al., 2016). Likewise, eccentric elbow flexions decreased ROM by only ~5% immediately post-exercise in untrained females (Lin et al., 2018) and similarly in males immediately (~10% peak decrease) (Chen et al., 2016), 24 h (~8% peak decrease) (Howatson & Van Someren, 2003), and 48 h (~7% peak decrease) (Lamb et al., 2019) post-exercise. These data are comparable to those reported by Chen et al. (2011) who showed that, in untrained males, maximal eccentric exercise performed with arm muscle groups induces greater muscle damage and decreases in ROM than when performed with leg muscle groups (-7.2% vs. -2.8%, respectively). However, others have documented greater reductions of up to 33% in ankle dorsiflexor ROM following leg-based resistance exercise in trained males and females (Casanova et al., 2018). These differences suggest an influence of training status on the exercise-induced change in ROM, which is evident for other markers of EIMD (Ertel et al., 2020; Vincent & Vincent, 1997). The change in ROM also appears to be affected by muscle contraction type, considering that 60 maximal unilateral elbow flexions incorporating concentric-eccentric (Hunt et al., 2021) or eccentric-only (Zainuddin et al., 2005) contractions reduced ROM by ~9% and ~30%, respectively in untrained males and females. Again, it is widely accepted that eccentric muscle contractions induce the severest damage (Allen, 2001; Armstrong, 1984; Ebbeling & Clarkson, 1989; Stauber, 1989). Less extensively examined is the impact of sex on ROM succeeding damaging exercise. Several studies have been conducted with both males and females; however, these did not analyse for sex differences (Casanova et al., 2018; Hunt et al., 2021; Zainuddin et al., 2005). Sex differences were analysed by West and colleagues (2020) who found that untrained males and females equally decreased knee joint ROM in response to 6 sets of 10 unilateral leg extensions. On the other hand, the relaxed arm angle of males and females was measured by Rinard et al. (2000) before and for 7 days after a bout of maximal eccentric elbow flexor exercise. Although no sex differences were apparent for 48 h after exercise, thereafter females had a significantly lower relaxed arm angle indicative of greater muscle stiffness and limited flexibility. Overall, the exercise-induced decrease in muscle flexibility and ROM varies in magnitude, which is perhaps due to the muscle group exercised, type of contraction, and familiarity with the exercise. Further research is needed before the impact of sex can be verified.

Muscle Soreness

Muscle soreness is a common consequence of eccentric or unaccustomed muscle contractions and generally peaks 48 h succeeding the exercise bout, hence the colloquial name 'delayed onset muscle soreness' or 'DOMS' (Damas et al., 2016_a). Subjective ratings of muscle soreness are typically measured with use of visual analogue/rating scales (VAS/VRS) or pressure algometry with the muscle either relaxed, flexed, or palpated and the participant in a standing, seated, or supine position. Muscle soreness can persist for ~4 days after exercise, though its severity and duration are highly variable (Damas et al., 2016_a). A large-scale cluster analysis conducted by Damas and colleagues (2016_a) suggested that the severity of soreness was related to the magnitude of muscle damage. By this means, young males who experienced the greatest reduction in elbow flexor MVC 1–5 days after eccentric exercise, i.e. high responders, reported the worst muscle soreness. Others, however, argue that muscle soreness is a poor reflection of EIMD (Nosaka et al., 2002_a), which is supported by studies that have failed to show a relationship between peak muscle soreness and immediate post-exercise reductions in maximal strength (Chen et al., 2020; Nosaka et al., 2002_a; 2002_b; Paschalis et al., 2005). It should be noted that sensations of soreness and pain are not only individual and subjective, but also can be influenced by present mood, hormonal status, and other physiological variables (Melzack, 1982). These factors may not influence other, more objective, markers of EIMD in the same way and could therefore explain the discordant relationship of muscle soreness and other EIMD outcomes.

The exact mechanisms underpinning muscle soreness are currently unknown. It appears that the development of muscle soreness follows an alternative mechanism of action to other symptoms of EIMD, given that strategies that attenuate muscle soreness do not necessarily preserve muscle strength (Farup et al., 2014; Philpott et al., 2018) and *vice versa* (Buckley et al., 2010; Cockburn et al., 2008; Naclerio et al., 2020). It has been postulated whether exercise-induced muscle soreness and pain are less so attributed to mechanical myofibre damage and more to metabolic events and disruption of the extracellular matrix (Stauber et al., 1990). There is an apparent time-course discord between the immediate post-exercise mechanical damage (indicated by reduced ROM, MVC etc.) and the delayed onset of muscle soreness. Further, muscle soreness induced by maximal eccentric contractions has been poorly correlated to strength loss (r = -0.346), while the relationship between strength loss and ROM, limb circumference, or [CK], albeit not strong, is more apparent (r = -0.494 to 0.449) (Damas et al., 2016_a). These data perhaps refutes a mechanical aetiology of muscle soreness.

A plausible metabolic mechanism relates to the sensitisation of pain receptors located within the muscle fascia, termed nociceptors (Marchettini et al., 1996). Following EIMD, an increase in cell membrane permeability and disturbed calcium homeostasis may trigger the calcium-mediated degranulation of mast cells. Upon their degranulation, mast cells release histamine, a known algesic that can sensitise nociceptors and increase pain sensations (Stauber et al., 1990). This concept holds strong considering the effectiveness of pain medications that block the degranulation of mast cells by calcium (Casale, 1988; Flacco et al., 1989; Amelink et al., 1990). Further, supplementation with caffeine, which increases calcium influx into muscle (Martonosi, 1984), has been documented to reduce sensations of soreness following eccentric exercise (Chen et al., 2019; Hurley et al., 2013). Therefore, myofibre membrane instability and calcium dysregulation may be key mediators of exercise-induced muscle soreness, although there is also a potential role of inflammation.

Systemic inflammation has been accepted as being poorly connected to exercise-induced muscle soreness (Donnelly et al., 1990; Kanda et al., 2013; Malm, 2001; Ms et al., 2020; Pizza et al., 1999; Uchida et al., 2009), although local inflammation, arising from structural deformation of myofibre membranes, could be a driving factor. Encouraging a local anti-inflammatory response within the muscle fascia and preserving cell membrane integrity was proposed by Philpott and colleagues (2018) as the key to attenuating exercise-induced muscle soreness, though this needs further investigation. Additionally, muscle soreness may be a consequence of altered behaviour of the muscle fascia connective tissue, which has been documented after eccentric exercise (Peñailillo et al., 2015; Wilke & Behringer, 2021). There is evidence of increased fascia thickness (Tenberg et al., 2021) and connective tissue breakdown (Brown et al., 1997; Mavropalias et al., 2021) after eccentric cycling and resistance exercise in males and females, though these changes are not always related to muscle soreness. The density of nerve endings is greater in the fascia than the muscle itself (Highstead et al., 2005) and, by this means, the fascia has been associated with greater pain sensations than muscle tissue in response to chemical (Deising et al., 2012; Schilder et al., 2014) or electrical (Schilder et al., 2016) stimulation, which was intensified following exercise (Gibson et al., 2009). Further, supplementation with gelatine or collagen increased connective tissue synthesis (Shaw et al., 2016) and alleviated symptoms of muscle soreness following exercise (Clifford et al., 2019), although more confirmatory studies are needed. Therefore, investigating the properties of connective tissue during exercise and their relationship with muscle soreness seems logical moving forward.

Overall, current data suggest that the mechanisms of exercise-induced muscle soreness are related to impaired cell membrane integrity and calcium dysregulation, local inflammatory responses, and/or altered connective tissue properties. While further inspection of these mechanisms is necessary, the contribution of systemic inflammation and mechanical myofibre damage to the development of muscle soreness likely can be ruled out.

2.3.3. Mechanisms of Muscle Repair and Regeneration

Succeeding muscle damage, the inflammatory response serves to remove damaged or necrotic cells and initiate vital muscle repair and adaptation processes. Although inflammation has long been considered a detrimental phenomenon, it is gaining acceptance that inflammation is necessary for effective muscle regeneration (Chazaud, 2016; Urso, 2013). Initially after muscle damage, various immune cell types, including neutrophils, eosinophils, T regulatory lymphocytes, CD8 T lymphocytes, and mast cells, are activated and infiltrate the muscle (Burzyn et al., 2013; Castiglioni et al., 2015; Côt́e et al., 2008; Teixeira et al., 2003; Zhang et al., 2014). Neutrophils are first to enter the muscle after exercise (Fielding et al., 1993) and are primarily involved in the phagocytosis of necrotic myofibres (Pizza et al., 2005), although can also produce cytolytic and cytotoxic molecules that may aggravate existing muscle damage (Nguyen & Tidball, 2003). Yet, animal models have demonstrated that depletion of neutrophils prior to muscle damage compromises muscle regeneration (Teixeira et al., 2003), which suggests that neutrophils hold an overall positive role in muscle health. Additionally, preventing monocyte recruitment to the injured site supressed macrophage activation and encumbered muscle regeneration (Lu et al., 2011). Macrophages possess a dominant role throughout all stages of muscle repair and are recruited within a few hours of muscle damage (Arnold et al., 2007; Perdiguero et al., 2011; Wang et al., 2014). Initially, macrophages serve as pro-inflammatory agents by stimulating the release of IL-1β, IL-6, and TNF-α and phagocytising necrotic cells and tissue debris (McLennan, 1996). The phagocytosis of necrotic myofibres triggers a switch in the phenotype of macrophages from pro- to anti- inflammatory agents, which then stimulate increased expression of IL-10 and growth factors and dampen pro-inflammatory cues (Arnold et al., 2007; Varga et al., 2013). Macrophages also support myofibril growth by stimulating their proliferation, while inhibiting their fusion and apoptosis, and are therefore considered essential to muscle regeneration (Chazaud et al., 2003; Sonnet et al., 2006). Macrophages themselves will proliferate, and peak concentrations of pro- and anti-inflammatory agents may respectively be measured within 12–48 h and 3–7 days following

EIMD, irrespective of the type of exercise (Chazaud, 2016; McLoughlin et al., 2003; Paulsen et al., 2013; Pizza et al., 2002; Tsivitse et al., 2003). The degree of macrophage infiltration is related to the severity of EIMD, as classified by the reduction in maximal muscle force following exercise, although macrophage numbers will remain elevated until tissue remodelling is complete (Paulsen et al., 2012).

Many peri-exercise nutritional, physiotherapeutic, and pharmaceutical strategies, such as antioxidant and polyphenol supplementation, cryotherapy, and non-steroidal anti-inflammatory drug (NSAID) administration hold the aim of reducing inflammation. However, as concluded by Chazaud (2016), supressing the initial pro-inflammatory phase can impede the subsequent stages of muscle regeneration, resulting in incomplete muscle repair. For example, the daily administration of NSAIDs to endurance trained males for 4 days before and 8 days after a 36 km run attenuated the post-exercise activation and proliferation of satellite cells (Mackey et al., 2007). Likewise, a peri-exercise intramuscular infusion of NSAIDs or placebo was received by endurance trained males for 7.5 h and 200 maximal eccentric leg extensions were performed. Immunohistochemistry analysis of muscle biopsy samples revealed that compared to the untreated leg, the leg infused with NSAIDs demonstrated an attenuated increase in satellite cell number 8 days post-exercise (Mikkelsen et al., 2009). As discussed below, satellite cells are key players in muscle remodelling and growth and hence these data indicate that muscle repair is impaired when the inflammatory response is obstructed, at least in males. To conclude, post-EIMD inflammation is likely beneficial for subsequent muscle regeneration and by extension, interfering with the initial pro-inflammatory phase with use of nutritional, physiotherapeutic, or pharmaceutical practices may not be pragmatic. Instead, Chazaud (2016) proposed that it is most appropriate to enhance the anti-inflammatory phase via timely post-exercise administration of anti-inflammatory agents, though this needs supporting investigation.

Succeeding the acute inflammatory cascade, skeletal muscle remodelling is dependent on resident stem cells, termed satellite cells. Satellite cells can be activated in response to physiological or metabolic stress, such as inflammation, oxidative stress, hypoxia, tissue injury, and exercise (Abreu et al., 2017). Unlike muscle degeneration, satellite cell activation is not restricted to the local site of damage. Satellite cells from an undamaged region of a myofibre also are activated, proliferate, and migrate to the damaged site on the same myofibre (Schultz et al., 1985). Then, 24–48 h following muscle damage, satellite cells rapidly express myogenic transcription factors (Cooper et al., 1999; Cornelison & Wold, 1997), which play key roles in

myoblast differentiation and proliferation (Gayraud-Morel et al., 2007; Yablonka-Reuveni et al., 1999). Following differentiation, satellite cells fuse to either existing damaged myofibres or each other to form new myofibres and the donated myonuclei translocate to the periphery of the fibre. These newly formed myofibres may then hypertrophy and are morphologically and functionally indifferent to undamaged fibres (Yin et al., 2013). Any excess satellite cells will replenish the satellite cell pool, as a diminished pool compromises muscle regeneration (Sambasivan et al., 2011).

Exercise is a potent stimulator of satellite cell activation. Several studies have been conducted with young, untrained males and these have showcased that maximal eccentric leg extensions can increase myofibre satellite cell content by up to 184% from pre- to 72 h post-exercise, which can remain elevated for up to 8 days (Crameri et al., 2007; Crameri et al., 2004; McKay et al., 2009; McKay et al., 2010; O'Reilly et al., 2008). The resistance exercise-induced activation of satellite cells has been related to muscle fibre type (increased activation at 96 h post-exercise in type I but not type II fibres in middle-aged, overweight/obese males and females) (Pugh et al., 2018), age (greater activation in young compared with older males; 141% vs. 51% increase 24 h post-exercise) (Dreyer et al., 2006), and sex (greater activation in young, untrained females compared with males) (Luk et al., 2019). Conversely, sex differences in the satellite cell response to 14 × 5 eccentric elbow flexions performed by recreationally active young adults were not identified by Paulsen et al. (2010), and so further studies are needed to confirm the impact of sex on satellite cell regulation. Likewise, the impact of hormonal status on satellite cell activation is poorly understood, mainly due to a lack of studies conducted with females, and specifically with consideration for MC phase, HRT use, and menopausal status (Oxfeldt et al., 2022).

2.3.4. Skeletal Muscle Adaptations – The Repeated Bout Effect

It is well appreciated that the changes in muscle histology, functional capacity, intramuscular protein leakage, and soreness experienced after unaccustomed exercise are attenuated with a repeated bout of a similar exercise performed within several weeks, termed the RBE (Fridén et al., 1983; Hough, 1902; Schwane & Armstrong, 1983). There is evidence to suggest that the RBE may be induced by eccentric contractions generating only 20% of maximal force and may be sustained for up to 3 weeks following the first exercise bout (Chen et al., 2012). However, it appears that the magnitude of the RBE is proportional to the intensity of the first exercise bout. Accordingly, a series of maximal eccentric contractions of the elbow flexor were

performed by untrained males 2–3 weeks after an initial series of eccentric contractions of the same arm performed at 100%, 80%, 60%, or 40% of maximal intensity. The protection against muscle damage offered by the first exercise bout on the second existed in an intensity-dependent manner (100% > 80% > 60% > 40%) (Chen et al., 2007). This effect may be attributed to the severity of muscle damage induced by high-intensity contractions, although importantly, severe muscle damage is seemingly not a prerequisite to the RBE (Chen et al., 2012). The magnitude of the RBE appears to differ among markers of muscle damage with the greatest protection being offered to myofibre membrane permeability, then to muscle soreness, and the least protection offered to maximal strength (Chen et al., 2016), which indicates that sarcolemma damage is notably reduced during repeated exercise. Other factors influencing the RBE include training status, i.e. greater protection in untrained than trained, and the muscle exercised, i.e. greater adaptation in the elbow flexor than the knee extensor (Nosaka & Aoki, 2011). Additional studies are needed to confirm the influence of sex on the RBE; however, no differences between recreationally active males and females in the protection against maximal torque loss following a repeated bout of eccentric dorsiflexor contractions were identified by Bruce et al. (2021).

The mechanisms of the RBE are not well understood, but are thought to be associated with neural, mechanical, and cellular adaptations. One proposal is that muscle adaptations are driven by an increasing number of sarcomeres in series within a myofibre (Lynn & Morgan, 1994; Morgan, 1990) which, although reversible, can occur rapidly (Williams & Goldspink, 1973). As a result, at any given muscle length, the sarcomere length would be shorter and therefore, the risk of sarcomere overextension beyond the point of actin-myosin overlap is reduced. Alternatively or additionally, a shift in the sarcomere length-tension curve (Gordon et al., 1966) may signify adaptation to EIMD. Due to sarcomere disruption, unaccustomed exercise causes a temporary shift in the optimum muscle length (Jones et al., 1997), followed by a second delayed shift, which represents adaptation by increased sarcomere number without changing tendon length (Brockett et al., 2001; Lynn et al., 1998). By this means, the contracting muscle may extend to longer lengths before a loss of tension occurs (Proske & Morgan, 2001a). Another proposition is that muscle adaptations are driven by the central nervous system by means of increased motor unit firing synchronicity, enhanced efficiency of motor unit recruitment, increased recruitment of type I fibres, and better workload distribution among fibres (Nosaka & Aoki, 2011). Using intramuscular electromyography, an increase in motor unit synchronisation was demonstrated up to 7 days following eccentric exercise (Dartnall et

al., 2011), which may enhance force production. These neural adaptations might explain why strength improvements can be made disproportionately to changes in muscle mass (Moritani, 1979). Nevertheless, a limited number of studies have investigated short- and long-term neural adaptations after eccentric exercise (Hyldahl et al., 2017). Establishing the mechanisms underpinning the RBE may shed light on those factors influencing the magnitude of its effects.

Due to the RBE, it could be argued that EIMD is only an issue for the first bout of exercise, as thereafter, symptoms that may impact exercise performance will be reduced. However, current resistance exercise recommendations prescribe progressive overload (ACSM, 2009) whereby the exercise type, set number, repetition range, load etc. are frequently changed. By this means, exercising individuals of any calibre are regularly exposed to "new" exercise stimuli and hence to EIMD. This highlights the importance of finding effective and sustainable strategies for the management of EIMD.

2.3.5. Sex Differences in Muscle Damage and Repair

The question as to whether sex differences exist in the muscle damage response to eccentric exercise has been heavily debated among researchers (Hubal & Clarkson, 2009; Pizza, 2009; Tiidus & Enns, 2009). Observed sex differences have been attributed to the role of oestrogen in maintaining cell membrane stability and inhibiting leukocyte infiltration (Tiidus, 2003). As shown in rat quadricep femoris muscle, damage induced by eccentrically biased downhill running was of greater severity, evidenced by increased sarcolemma permeability, in males than females (Komulainen et al., 1999). Furthermore, male rats supplemented with oestrogen had attenuated intramuscular CK leakage during exercise recovery compared to untreated rats; the response to which was augmented with increased duration of oestrogen supplementation (Clarkson & Sayers, 1999). These observations, among others (Amelink & Bär, 1986; Amelink et al., 1990; Bär et al., 1988; St. Pierre Schneider et al., 1999) led researchers to investigate, in human participants, the impact of oestrogen on skeletal muscle damage. However, while studies conducted with animals can be tightly controlled, it is challenging to isolate the impact of specific hormones in humans due to, for instance, hormone interactions and ethical constraints with performing ovariectomies or issuing hormone replacement therapy (HRT) and hormonal contraceptives (HCs) in a blinded fashion. As such, it is not surprising that the outcomes obtained from animal studies are not always mirrored in human studies that have attempted to uncover the effects of oestrogen and sex on skeletal muscle damage.

Human studies have compared muscle responses to damaging exercise between males and females, females with or without HRT use, and during different phases of the menstrual cycle (MC). So far, findings are equivocal. For instance, premenopausal HC users (Carter et al., 2001) and postmenopausal HRT users (Dieli-Conwright et al., 2009) had lower post-exercise CK responses relative to HC and HRT non-users, respectively. However, in another instance, HC users showed greater post-exercise [CK] elevations than non-users (Minahan et al., 2015). Therefore, synthetic female hormones may or may not help protect myofibre membranes during exercise. Further, compared to males, females have experienced an attenuated (Stupka et al., 2000) or heightened (MacIntyre et al., 2000) inflammatory response; lower muscle soreness (Kerksick et al., 2008); greater strength impairment (Fredsted et al., 2008; Sewright et al., 2008); and blunted [CK] elevation (Minahan et al., 2015; Sewright et al., 2008) following eccentric exercise. Conversely, other studies have failed to identify sex differences in post-exercise strength loss, muscle soreness, or intramuscular protein leakage (Eston et al., 2000; Hubal et al., 2008; MacIntyre et al., 2000; Rinard et al., 2000; Sayers & Clarkson, 2001). Disagreements in these data have been attributed to methodological limitations in study design, such as lack of control for hormonal status; poor measures of muscle damage; and inequitable exercise protocols (Hubal & Clarkson, 2009; Pizza, 2009). In accordance, the research design of Dannecker and colleagues (2012) aimed to mitigate these limitations by first, obtaining measurements from females during the follicular phase of the MC (although confirmation of MC phase was not gained through blood hormone concentration); second, by establishing the intensity of the exercise protocol based on eccentric strength, not concentric strength; third, by assessing muscle soreness of the exercised limb during rest, movement, and maximal strength testing; and fourth, by controlling participants' diet and therapeutic behaviours. By this means, in response to a series of maximal eccentric elbow flexor contractions, young, untrained males and females experienced comparable changes in subjective muscle pain, PPT, swelling, and serum biomarkers. In support, a recent systematic review with meta-analysis revealed marginal difference between males and females in maximal voluntary isometric torque loss of the lower and upper extremities (1.3% and 6.5% weighted mean difference, respectively) immediately following EIMD (Morawetz et al., 2020). Further, self-perceived muscle soreness was comparable between sexes in all studies reviewed. On the other hand, in six of the nine studies analysed, males experienced a significantly larger post-exercise [CK] peak relative to females, with no sex differences in the time-course of peak [CK] (Morawetz et al., 2020). Therefore, the influence of sex and oestrogens on EIMD may be specific to individual muscle damage markers.

Oestrogenic hormones and sex perhaps have a greater influence on muscle repair and adaptation following EIMD. The involvement of oestrogens in post-injury inflammatory processes (section *2.3.3.*), specifically by attenuating leukocyte infiltration, has been frequently argued in animal models (Enns et al., 2008; St. Pierre Schneider et al., 1999; Tiidus, 2003; Tiidus & Bombardier, 1999; Tiidus et al., 2001). Yet, corroborating data from human studies are lacking, mainly because the effects of oestrogen *per se* cannot be isolated; hence, comparisons between sexes are made. For instance, a high-volume of eccentric contractions induced more severe neutrophil infiltration in females than males (MacIntyre et al., 2000). Likewise, immediately after a bout of downhill running, females had greater elevations in neutrophils, lymphocytes, monocytes, and total white blood cell count compared to males, who experienced a delayed (24 h post-exercise) inflammatory response (Wiecek et al., 2017). In a conflicting study by Stupka et al. (2000), males had a higher inflammatory cell count than females, though the inflammatory response induced by eccentric exercise was not significantly different between sexes. Albeit these data may have been influenced by HC use among females and the significantly higher dietary antioxidant intake in males compared to the females. Clearly in humans, unlike rodents, the relationship of sex/oestrogen and inflammatory muscle repair events is ambiguous.

Considering the importance of the post-damage inflammatory cascade in muscle repair and regeneration (Arnold et al., 2007; Varga et al., 2013), it may be postulated whether the diminution of leukocyte infiltration by oestrogen is indeed beneficial, or otherwise impeding, to muscle recovery. As such, a 'Goldilocks zone' for post-damage muscle inflammation and repair that may be achieved when oestrogen levels are 'normal' and not supraphysiological, as often used in experimental models, was proposed by Tiidus (2018). Further, faster recovery of muscle force concurrent with an augmented inflammatory response was demonstrated by Le and colleagues (2018) in ovariectomised mice under conditions of 'normal' oestrogen, relative to 'low' oestrogen. While these data support an optimal level of oestrogen for inflammatory-mediated muscle repair, other indices of muscle repair, such as satellite cell proliferation, may be augmented with supraphysiological levels of oestrogen (Mangan et al., 2014).

Several rodent studies have demonstrated a role of oestrogen in the post-damage activation and proliferation of skeletal muscle satellite cells (Collins et al., 2019; Enns et al., 2008; Enns & Tiidus, 2008; Mangan et al., 2014; Thomas et al., 2010; Velders et al., 2012). In one such study, ovariectomised rats were subjected to downhill running exercise with or without prior oestrogen supplementation. Exercise increased the number of myofibres containing activated

and proliferating satellite cells and these numbers were augmented with oestrogen treatment (Enns & Tiidus, 2008). Others have observed loss of satellite cells with oestrogen deficiency and inhibition of oestrogen receptors (ER) (Collins et al., 2019). ER-α and ER-β are thought to be mechanistically involved in the oestrogenic activation of satellite cells (Thomas et al., 2010; Velders et al., 2012). The exact signalling pathways by which oestrogen communicates with satellite cells are currently unknown, though the PI3-K pathway was placed by Mangan et al. (2014) as a central link between oestrogen and satellite cell activation.

A direct link between oestrogens and satellite cell activation has not yet been established in humans. Importantly, the nuclei expression of ER-α in human skeletal muscle was shown by Wiik and colleagues (2008) to not be a factor governing sex differences in satellite cell activation. Due to the methodological constraints of isolating the effects of oestrogen, a comparison of the muscle fibre satellite cell content between sexes and across different age groups may be used as a proxy. Cross-sectional analyses have found the number of satellite cells per muscle fibre of males and females of the same (Kadi et al., 2004) or different (Roth et al., 2000b) age to be comparable. The muscle satellite cell pool may diminish with age (Kadi et al., 2004; Renault et al., 2002), such that type II muscle fibres can contain 24% fewer satellite cells in older adults (≥50 y) compared to young (18–49 y) (Verdijk et al., 2014). However, others have refuted an age-dependent loss of satellite cells (Dreyer et al., 2006; Roth et al., 2000_b), which suggests that age-related changes in the sex steroid hormone milieu are not responsible for changes in satellite cell content. Unfortunately, most studies of this nature have not measured sex steroid hormone concentrations and so an indirect relationship with satellite cells cannot be inferred. Nonetheless, current data does not firmly suggest a sex- or an age-dependent activation of satellite cells within human skeletal muscle; indicating that post-injury muscle repair processes are similar between males and females.

Overall, whether sex differences exist in skeletal muscle damage and repair remains debatable. In animal models, female rodents typically suffer less muscle damage – likely due to an oestrogen-mediated attenuated increase in sarcolemma permeability – whereas the same response is less clear in humans. Varied EIMD responses between males and females and between females of different oestrogen status have been reported, which may be owing to limitations in methodological design. Oestrogens seem to enhance the repair of rodent muscle by reducing inflammation and stimulating satellite cell activation and proliferation; though in humans, there are no established effects of oestrogen nor sex on inflammatory events, satellite cell activation, or functional markers of muscle repair. Herein, sex comparative and sex-

specific research has been drawn upon where available; however, a paucity of female-focussed research has been highlighted and hence, justification for the need for female inclusive research is next provided.

2.4.0. Exercise Physiology and Nutrition Research with Females

Sport and exercise science research has heavily underrepresented female populations, who since 2014, comprised only 34% of research participants (Cowley et al., 2021). Nevertheless, the outcomes and practical recommendations derived from these studies are often assumed to be applicable to both sexes, which is likely, in many cases, a false assumption. Without research conducted with both sexes or replicated in females, it cannot be known whether the outcomes of current studies conducted with males can be extrapolated to females. Due to the physiological sex differences (*Figure 2.4*) that primarily stem from sex steroid hormone dissimilarities (see *Table 2.1* for a summary of the functions and normal ranges of sex hormones in males and females), it is unlikely that females will always respond comparably to males to nutrition and exercise stimuli. Females who adhere to nutrition and training guidelines stemming from research conducted with males may not reach their true potential, be that exercise performance, body composition adaptations, or general health. It follows that more high-quality female-focussed research, based on the current guidance for standards of practice for research with female participants (Elliott-Sale et al., 2021), is required to generate sex-specific nutrition and exercise recommendations.

Based on the currently available research using female participants, it is challenging to draw consensuses on topics due to inconsistencies in methodology and terminology, particularly relating to hormonal status (Cable & Elliott, 2004). Throughout the lifespan, females experience acute and chronic hormonal changes owing to the MC, HC use, HRT, pregnancy, and menopause, which influence several biological systems and processes [e.g. reproductive, vascular, nervous, skeletal, cardiovascular, immune, and metabolism (Boss et al., 2014; Hackney et al., 1994; Isacco et al., 2012; Sipilä et al., 2020; Somani et al., 2019; Wierman, 2007; Wright & Badia, 1999)] as well as physiological responses to exercise, as detailed in sections *3.4.3.* and *2.4.5*. However, research conducted with females has often failed to control for or report details of hormonal status, which can result in the grouping of non-homogenous participants and great variability between studies (Elliott-Sale et al., 2021). When researching females, it is therefore important to adapt study designs to accommodate these hormonal fluctuations (e.g. obtain measurements during the same MC phase within and between

participants), and to consistently define study samples based on hormonal status (e.g. pre, peri, or post- menopausal). Such uniformity will help inform nutrition and exercise recommendations and increase the confidence in which these recommendations can be applied to female sub-groups.

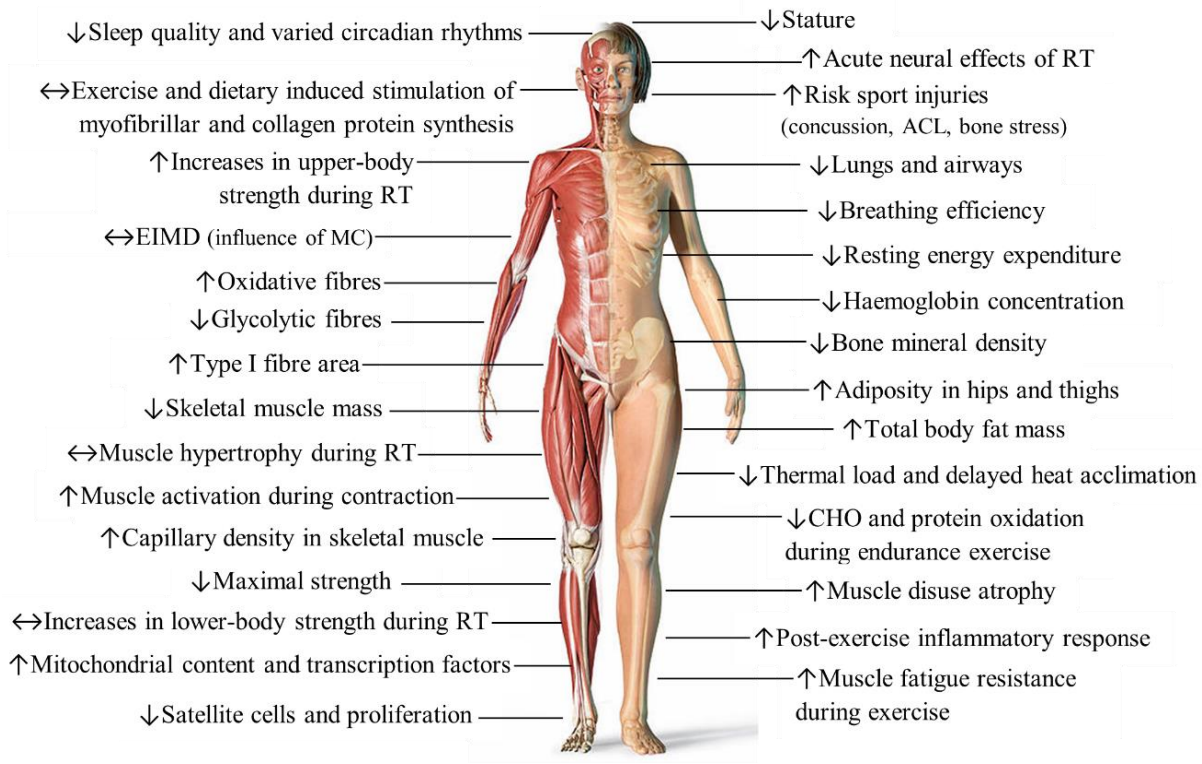


Figure 2.4. Physiological differences in females relative to males.

Arrows indicate that the variable is greater/more (↑),lower/less (↓), or similar (↔) in females compared with males. RT = resistance training; MC = menstrual cycle; ACL = anterior cruciate ligament; CHO = carbohydrate. Data from: Ansdell et al. (2020); Delmonico et al. (2005); Devries (2016); Dominelli et al. (2019); Dominelli et al. (2018); Hunter (2016); Ivey et al. (2000); Karastergiou et al. (2012); Lin et al. (2018); Makovey et al. (2005); Martel et al. (2006); Miller et al. (1993); Miller et al. (2006); Mong & Cusmano (2016); Nielsen et al. (2003); Roberts et al. (2020); Roepstorff et al. (2006); Rosa-Caldwell & Greene (2019); Staron et al. (2000); Wickham et al. (2021); Wiecek et al. (2017); Zeller et al. (2003).

Table 2.1. Sex steroid hormones – functions, normal ranges, and consequences of deficiency

Sex steroid hormone	Sex	Site of production / release	Functions	Normal ranges	Consequences of deficiency
Oestrogens (oestradiol, oestrone and oestriol)	Females	Ovaries and adipose tissue	Maintain sexual function and secondary sex characteristics; aid energy homeostasis; potential role in reducing muscle damage and inflammation; aid muscle repair by activation and proliferation of satellite cells; involved in gene transcription regulation; maintain endothelial and bone health (reduce osteoclast activity)	15-350 pg•mL^{-1} premenopausal <40 pg•mL^{-1} postmenopausal	Impaired skeletal maturation and lowered bone mass and mineral density (due to increased osteoclast activity); endothelial dysfunction (increased CVD risk)
	Males	Testes (20%), conversion from testosterone, and adipose tissue	Help modulate energy homeostasis; required for fluid resorption by efferent ductule epithelium; maintains sperm structure and motility	3.6-91 pg•mL^{-1}	Impaired skeletal maturation and lowered bone mass and mineral density; reduced epithelial height and impaired fluid reabsorption at the efferent ducts
Progesterone	Females	Ovaries and adrenal glands	Thermoregulation; modulates LH secretion; energy metabolism; promotes gene transcription of reproductive organ proteins; aids neurotransmitter function; essential for healthy pregnancy; mediates endometrium maturation; neurosteroid and interacts with GABA receptors to influence mood and cognition	<1-35 ng•mL^{-1} 100-300 ng•mL^{-1} during pregnancy	Oligo-/amenorrhoea; reduced fertility; symptoms of depression/anxiety; impaired blood glucose regulation
	Males	Testes and adrenal glands	Testosterone production; bone development; blood glucose regulation;	0.13-0.97 ng•mL^{-1}	Fatigue; poor muscle development; sexual

			thyroid hormone production; energy metabolism		dysfunction; increased prostate cancer risk
Testosterone	Females	Ovaries (25%), adrenal cortex (25%), and conversion from androstenedione (50%)	Maintain sexual function; support endothelial and arterial function; offer neuroprotective and anti-inflammatory effects in the brain; support musculoskeletal health	15–70 ng•dL^{-1}	Reduced bone mineral density; potentially increased risk of cardiovascular disease; polycystic ovary syndrome (when too high)
	Males	Testes (95%) and adrenal glands (5%)	Maintain sexual and reproductive function, muscle mass, and bone mineral density; precursor to oestradiol and 5α-dihydrotestosterone; inhibits GnRH, LH and FSH secretion via negative feedback	>300 ng•dL^{-1} Total >65 pg•mL^{-1} Free	Impaired sexual function; muscle atrophy; increased adiposity; osteoporosis; hair loss; increased comorbidity risk
Luteinising hormone (LH)	Females	Anterior pituitary gland	Released in response to the pulsatile release of GnRH; stimulates the release of steroids from the ovaries; stimulates ovulation and the release of progesterone; essential for ovarian follicle growth	0.6–56.6 IU•L^{-1} 14.2–52.3 IU•L^{-1} postmenopausal	Impaired sexual maturation; oligo-/amenorrhoea; infertility
	Males	Anterior pituitary gland	Released in response to the pulsatile release of GnRH; stimulates Leydig cells to produce testosterone	1.2–7.8 IU•L^{-1}	Decreased androgen production; reduced spermatogenesis; impaired sexual maturation; high concentration indicates hypogonadism

Follicle stimulating hormone (FSH)	Females	Anterior pituitary gland	Released in response to the pulsatile release of GnRH; stimulates the release of steroids from the ovaries; promotes the conversion of androgens to oestrogens; necessary for follicular growth	4.5–21.5 IU•L^{-1} 25.8–134.8 IU•L^{-1} postmenopausal	Delayed/incomplete sexual maturation; oligo-/amenorrhoea; infertility
	Males	Anterior pituitary gland	Released in response to the pulsatile release of GnRH; stimulates the Sertoli cells to support spermatogenesis; necessary for follicular growth	1.5–12.4 IU•L^{-1}	Delayed sexual maturation; high plasma concentration indicates hypogonadism

Premenopausal females refer to post-menarche adults (≥18 y); males refer to adults (≥18 y); CVD = cardiovascular disease; LH = luteinising hormone; GABA = gamma-aminobutyric acid; GnRH = gonadotropin-releasing hormone; FSH = follicle stimulating hormone. Data from: Cooke et al. (2017); Davis & Wahlin-Jacobsen, (2015); Decaroli & Rochira (2017); Enns & Tiidus (2010); Honour (2018); Israel & Schneller (1950); Livingston et al. (2017); McGee & Hsueh (2000); Mohamad et al. (2016); Oettel & Mukhopadhyay (2004); Rariy et al. (2011); Somani et al. (2019); Sowers et al. (2001); Taraborrelli (2015); Väänänen & Härkönen (1996); Vanderschueren et al. (2014).

2.4.1. Hormonal Fluctuation in Premenopausal Females

The adult female lifespan may be divided into two key stages based on reproductive status, i.e. premenopausal and postmenopausal. Menopause, as defined by the absence of menses for 12 months, is triggered by the loss of ovarian function and typically occurs between the ages of 40 and 60 years (Honour, 2018). There are notable differences in the hormone profiles of females during these life stages, due to dramatic changes in sex steroid hormones during menopause (Al-Azzawi & Palacios, 2009). Specifically, postmenopausal females secrete lower concentrations of oestrogen and progesterone and higher concentrations of follicle stimulating hormone (FSH) compared to premenopausal females (see *Table 2.1* for concentration ranges). As such, females with different menopausal status can experience variances in, for example, thermoregulation, energy metabolism, body composition, and adaptations to exercise (Bondarev et al., 2018; Charkoudian & Stachenfeld, 2016; Greendale et al., 2019; Isacco et al., 2012; Sowers et al., 2007). Likewise, the hormone milieu changes during pregnancy and lactation, which too can alter several physiological processes (Suresh & Radfar, 2004). It is beyond the scope of this review to discuss hormonal fluctuation and its physiological impact during all stages of the female lifespan. Therefore, unless specified, the term 'female' here refers to non-pregnant, non-lactating, premenopausal females.

2.4.2. The Menstrual Cycle

The female MC can be divided into 2–7 phases. Basic categorisation differentiates the follicular phase from the luteal phase, which are separated by the periovulatory phase and may be sub-divided into early, mid, and late phases (Elliott-Sale et al., 2021). While there is interindividual variability in the duration of each phase and total MC length (Fehring et al., 2006), the characteristics of each phase are, for the most part, common between eumenorrhoeic females. The onset of menstruation marks day one of the MC, which occurs in the early-follicular phase (days 1–4) and is associated with elevated secretion of FSH and low concentrations of oestradiol, progesterone, and luteinising hormone (LH) (Owen, 1975). During the late-follicular phase (days 5–11), plasma oestradiol and LH concentrations rise, while progesterone remains low. There is a surge in LH that triggers ovulation on day ~14 or 15 of the MC, during the periovulatory phase (days 12–15). Oestradiol concentration peaks during ovulation and remains heightened into the luteal phase (days 16–28), while LH and FSH return to normative levels (Fehring et al., 2006; Owen, 1975). In the early-luteal phase, progesterone concentration

surges, then subsides alongside oestradiol during the late-luteal phase, which triggers menstruation and the start of a new MC (Owen, 1975).

2.4.3. The Menstrual Cycle and Response to Exercise

Exercise performance and acute responses and chronic adaptations to exercise may be influenced by the MC, which can be examined through phase-based training, whereby exercise is performed predominantly in one MC phase. The systematic review with meta-analysis (n = 78 studies) by McNulty and colleagues (2020) investigated the impact of MC phase on endurance and resistance exercise performance. The authors concluded that exercise performance may be trivially impaired during the early-follicular phase (when oestrogens and progesterone are low) relative to all other MC phases. However, the studies included were mostly of low quality and vastly heterogenous, and so pooling of these data may not have been appropriate. Further, the overall effect size (ES) was very small (-0.06 [-0.16, 0.04]) and there were an equal number of studies significantly in favour of the early-follicular phase (n = 6) and other phases (n = 6). While the potential for exercise performance to be reduced during the early-follicular phase may have implications for elite athletes, it is unlikely relevant for recreational exercisers.

Exercise performance is perhaps not influenced by MC phase directly, but rather is a secondary consequence of hormonal fluctuation impacting other physiological processes. For instance, progesterone has thermogenic properties (Israel & Schneller, 1950) and the surge in progesterone release during the early-luteal phase triggers a ~0.4 °C rise in resting core body temperature (Coyne et al., 2000; Kattapong et al., 1995; Parry et al., 1997; Zhang et al., 2020), which can subsequently elevate resting energy expenditure (Bisdee et al., 1989; Pelkman et al., 2001; Solomon et al., 1982). However, the exercise-induced increase both in core and skin temperature is greater during the follicular phase compared to the luteal phase (Grucza et al., 1993), which may have implications for perceived exertion and exercise performance. Furthermore, it is possible that the negative symptoms often experienced during menstruation [e.g. abdominal pain, headache, backache, fatigue, psychological changes (Schoep et al., 2019)] may hinder females in reaching their maximum potential during assessments of exercise performance. Therefore, hormonal fluctuation during the MC may impact exercise performance indirectly via other hormone-regulated processes, which should be considered and controlled for where possible.

It has been suggested that during low-oestrogen phases of the MC, females may be more vulnerable to acute EIMD (Romero-Parra et al., 2021_a), which has been attributed to the protective effects of oestrogen on muscle cell membranes (Amelink & Bär, 1986; Amelink et al., 1990; Bär et al., 1988; Clarkson & Sayers, 1999; St. Pierre Schneider et al., 1999; Tiidus, 2003) (see section *2.3.5*). In their meta-analysis (n = 19 studies), Romero-Parra et al. (2021_a) demonstrated that during low-oestrogen phases (early-mid-follicular) females reported more severe post-exercise muscle soreness and suffered greater reductions in strength than during high-oestrogen phases (mid-luteal). Post-exercise [CK] was not influenced by MC phase, which suggests that oestrogen does not help maintain muscle cell membrane integrity. However, of the 19 studies included, 12 studies were not specifically designed to examine EIMD between different MC phases and hence only reported data obtained during one MC phase. This reason, as well as the varied training statuses of participants, likely contributed to the large heterogeneity observed in the meta-analysis. Further research that compares across multiple MC phases is needed to confirm the impact of hormonal fluctuation on EIMD. Although, owing to the strong evidence of oestrogenic protective effects on muscle damage in rodents (Amelink & Bär, 1986; Amelink et al., 1990; Bär et al., 1988; Clarkson & Sayers, 1999; St. Pierre Schneider et al., 1999; Tiidus, 2003), it seems wise to consider hormonal status when conducting EIMD research with humans.

Chronic training adaptations, such as skeletal muscle hypertrophy and strength gains, also may be influenced by the MC. Oestrogen possesses anabolic properties (Hansen, 2018) and in this regard, the anabolic potential of skeletal muscle may be heightened when oestrogen secretion is dominant in the late-follicular phase. Accordingly, the acute anabolic response to 1 h of unilateral leg kicking exercise (67% maximum workload) performed by untrained eumenorrhoeic females either in the follicular or luteal phase was documented by Miller and colleagues (2006). Exercise in both phases triggered comparable increases in the synthesis rates of muscle collagen and myofibrillar protein, indicating that progesterone does not increase acute protein catabolism, as previously suggested (Kriengsinyos et al., 2004; Lamont et al., 1987; Lariviere et al., 1994). However, over time, progesterone may influence muscle protein accretion, as differences in muscle hypertrophic adaptations with phase-based training have been revealed (Thompson et al., 2020). For instance, performing several months of leg-based resistance training during the follicular phase (when progesterone is low) resulted in greater enlargement of muscle cross-sectional area and superior strength gains compared to when luteal-based (Sung et al., 2014; Wikström-Frisén et al., 2017) or regular (Reis et al., 1995)

resistance training was performed. Conversely, equal strength gains and hypertrophy of the elbow flexor muscles following 12 weeks of follicular- or luteal- based resistance training were reported by Sakamaki-Sunaga et al. (2016). Therefore, it cannot be confirmed whether phase-based training is advantageous to resistance exercise adaptation. However, in a real-world setting, the feasibility and practicality of phase-based training may be questioned and limiting exercise conduct to approximately half of the month could interfere with other benefits of exercise, for example weight loss.

At this stage, it is unclear whether the MC can significantly affect exercise performance, EIMD, or training adaptations. Notwithstanding, given the known fluctuations in sex steroid hormones across the MC, it is regarded important to document and consider the MC phase in which primary measures are conducted in female participants. Future exercise physiology research with methodological considerations for females (Elliott-Sale et al., 2021) may help establish whether the MC is contributing to sex differences in the responses to exercise and nutrition stimuli.

2.4.4. Hormonal Contraceptives

Hormonal contraceptives are used by almost 50% of athletic and non-athletic female populations, with the oral contraceptive pill (OCP) being the most preferred method (Martin et al., 2018). Hormonal contraceptives provide exogenous synthetic progesterone (progestin) alone or in combination with synthetic oestrogen (ethinyl oestradiol), which consequently alters the sex steroid milieu; downregulates the hypothalamic-pituitary-ovarian axis via a negative feedback loop; and interferes with the natural MC (Elliott-Sale et al., 2020). Hormonal contraceptives are typically used for 21 days (active phase), followed by 7 withdrawal days (withdrawal phase), thereby creating "pseudo phases" that mimic a natural MC (Rechichi et al., 2009). During the active phase, HCs provide a constant rate of oestradiol and progestin (monophasic); constant rate of oestradiol and varied rate of progestin (biphasic); or varied rates of oestradiol and progestin (triphasic). Hormonal contraceptives prevent ovulation by supressing the follicular phase rise in endogenous oestrogens, LH, and FSH (Pelkman et al., 2001; Steward et al., 2016). Therefore, HC users have lower levels of endogenous oestrogens and progesterone compared to naturally menstruating females (Fleischman et al., 2010). The exogenous supply of progestin provided by HCs can interfere with thermoregulation (Kattapong et al., 1995; Rogers & Baker, 1997; Wright & Badia, 1999), resting energy expenditure (Cagnacci et al., 2006; Pelkman et al., 2001; Steward et al., 2016), exercise

performance (Elliott-Sale et al., 2020), and body composition changes in response to exercise training (Myllyaho et al., 2018; Romance et al., 2019). Therefore, it is important to consider HC use, including the type of HC, in exercise physiology research and it may be pragmatic to subgroup or test for differences between HC users and non-users.

2.4.5. Hormonal Contraceptives and Response to Exercise

The impact of OCP use on endurance and resistance exercise performance was systematically reviewed by Elliott-Sale and colleagues (2020). Exercise performance during the early-follicular phase of naturally menstruating females and the pseudo-early-follicular phase (i.e. OCP withdrawal week) of OCP users was compared in a meta-analysis (n = 18 studies). A small overall effect (0.18 [-0.02, 0.37]) was found in favour of naturally menstruating females over OCP users for exercise performance, irrespective of exercise type, with no individual study reporting significant effects. Comparing all other natural MC phases (days 6–28) with OCP consumption days (8–28) produced a very small overall effect (0.13 [-0.05, 0.28]; n = 24 studies) in favour of naturally menstruating females. These outcomes suggest that OCP use does not drastically lower exercise performance, although these data may be subject to bias, as 83% of the included studies were rated as very low-moderate quality. Furthermore, the impact of other forms of HCs, which can provide varied amounts of exogenous hormones, on exercise performance has not been reviewed to date.

Due to their impact on the female hormone milieu, HCs can alter acute muscle damage responses to exercise (Thompson et al., 2020). Several studies have examined the muscle damage response to exercise performed in the early-follicular phase of the MC (low oestrogens and progesterone) in naturally menstruating females compared with the pill withdrawal phase (low exogenous oestradiol and progestin) in habitual OCP users. For instance, 48–72 h following a series of unilateral eccentric elbow flexions, isometric MVC was reduced to a greater extent in untrained OCP users than non-users (Savage & Clarkson, 2002). Likewise, it was found by Mackay et al. (2019) that untrained females who habitually used OCPs suffered a 15–18% greater loss of maximal isometric strength 72–96 h after high-intensity eccentric cycling performed during the pill withdrawal phase (day 1–2) compared with naturally menstruating females during the early-follicular phase (day 1–2). These observations indicate that the higher secretion of endogenous oestrogens in naturally menstruating females relative to OCP users may exert protective effects against muscle function following EIMD, although this is not always the case (Hicks et al., 2017; Minahan et al., 2015).

OCP users appear to be at increased risk of cell membrane disruption following muscle damaging exercise (Hicks et al., 2017; Minahan et al., 2015; Roth et al., 2001). Recreationally active, naturally menstruating females experienced attenuated CK activity succeeding leg-based eccentric resistance exercise performed during the early-follicular (Minahan et al., 2015; Roth et al., 2001) or ovulatory (Hicks et al., 2017) phase compared with the hormonally-equivalent OCP phases. These studies reported higher serum concentration of oestrogens in the naturally menstruating females than OCP users, which supports previous observations of oestrogenic effects on membrane stability (Amelink & Bär, 1986; Amelink et al., 1990; Bär et al., 1988; Clarkson & Sayers, 1999; St. Pierre Schneider et al., 1999; Tiidus, 2003). However, when the CK response to eccentric back squat exercise was examined in resistance trained females during different OCP cycle phases, [CK] elevations were greater during the OCP withdrawal phase (~day 4; low exogenous oestradiol) compared with the active phase (~day 12; high exogenous oestradiol) (Romero-Parra et al., 2021_b). Given that post-exercise elevations in serum myoglobin and lactate dehydrogenase were indifferent between groups, the observed CK response is potentially due to intraindividual variability (Chrismas et al., 2018) and not to hormonal status. Furthermore, as found by Sim and colleagues (2015), hormonal variation across the OCP cycle did not impact the increase in serum IL-6 in recreationally active females following high-intensity treadmill running, indicating that OCP use does not affect acute inflammatory responses to tissue injury.

The impact of HCs on exercise-induced muscle soreness is confounding. In the study by Thompson et al. (1997), recreationally active habitual OCP users and non-users performed 50 min of bench stepping exercise during the pseudo-luteal and mid-luteal phases, respectively. Forty-eight hours after exercise, the OCP users experienced attenuated soreness upon palpation and movement of the quadricep muscles, but comparable soreness of the hamstring, glute, and calf muscles, compared with OCP non-users. These findings are in agreement with Roth et al. (2001) who documented lower resting muscle soreness in OCP users, though in disagreement with Mackay et al. (2019) who observed higher muscle soreness during bodyweight squat and delayed recovery of PPT in OCP users. Additionally, several others have reported no differences in exercise-induced muscle soreness between OCP users and non-users (Hicks et al., 2017; Savage & Clarkson, 2002) and between OCP active and withdrawal phases (Romero-Parra et al., 2021_b). At this stage, it cannot be ascertained whether hormonal variability due to HC use impacts the muscle soreness response to exercise.

Overall, naturally menstruating females may suffer less EIMD than OCP users, which is potentially due to endogenous oestrogens exerting protective effects on skeletal muscle. However, there is currently not enough evidence to suggest that OCP use is detrimental to muscle health and recovery following exercise. The limited studies conducted to date have investigated several different MC and OCP phases, with use of a range of OCP types, in diverse female populations; thus, it is unsurprising that outcomes are inconsistent. Furthermore, the impact of other forms of HCs (e.g. implant, intrauterine devices, injections, rings), which can provide varied amounts and combinations of hormones, on EIMD is unknown. If, upon further research, significant differences in EIMD between HC users and naturally menstruating females are identified this may broaden differences between sexes. Therefore, it is important to consider and report HC status, especially when comparing females with males.

Hormonal contraceptives have shown to limit the anabolic potential of skeletal muscle (Hansen et al., 2008; 2009; 2011), which could have implications for chronic training adaptations, such as muscle hypertrophy and strength improvement. Compared to naturally menstruating females during the early-follicular phase (low oestrogen and progesterone), OCP users in the active phase had significantly lower synthesis rates of tendon and muscle collagen and a blunted increase in collagen synthesis rate in response to exercise stimuli (Hansen et al., 2009). These authors later demonstrated that resting and post-exercise fractional synthesis rates of myofibrillar proteins also are inhibited by OCP use (Hansen et al., 2011). The anabolic response was dependent on the OCP type, with a higher provision of oestradiol (35 vs 30 µg) being associated with superior protein anabolism. These findings suggest that the supply of synthetic hormones and consequent reduction in endogenous hormone secretion downregulates the muscle protein synthetic machinery; although, whether this translates to blunted muscle adaptation over time is less clear.

To determine the effect of HCs on chronic exercise adaptation, several studies have compared changes in body composition and muscle function outcomes between HC users and non-users during exercise intervention. Muscle adaptations during 10 weeks of leg-based progressive resistance training were examined by Dalgaard et al. (2019) in untrained naturally menstruating females and habitual OCP users. Exercise training induced comparable increases in dynamic and isometric muscle strength and muscle tendon cross-sectional area in both groups. However, the enlargement of muscle cross-sectional area was superior to naturally menstruating females only in OCP users receiving a larger daily dose of oestradiol (30 vs 20 µg). This difference was predominantly due to a significant enlargement of type I muscle fibres in OCP users, which

remained unchanged in non-users during the intervention. These data imply that OCPs supplying higher amounts of oestradiol (30 µg) impact training-induced muscle hypertrophy in a fibre-type dependent manner. Nonetheless, it should be noted that Dalgaard et al. (2019) did not control for MC or OCP phase (i.e. hormonal status) in their measurements. Also, the OCP users had a significantly higher daily protein intake relative to body mass than the naturally menstruating females, which is known to enhance muscle hypertrophic responses to resistance training (Longland et al., 2016). Due to these confounding factors, the influence of OCP use on exercise adaptations cannot be confirmed by this study. Conversely, resistance trained females were prescribed a high protein diet [2 grams per day per kilogram of body mass ($g \bullet d^{-1} \bullet kgBM^{-1}$)] alongside 4 $d \bullet wk^{-1}$ of whole-body resistance training for 8 weeks by Romance et al. (2019). Habitual OCP (mono- or tri- phasic) users were assessed for muscle function and dual-energy X-ray absorptiometry (DXA)-derived body composition during the active pill phase, while naturally menstruating females were assessed during the late-follicular phase at baseline and post-intervention. The improvement in dynamic strength was unaffected by OCP use, although total body mass and fat-free mass (FFM) were increased to a greater extent in OCP users than non-users. In contrast, body composition did not change during 10 weeks of high-intensity combined strength and endurance training in recreationally active females using a range of HCs (combined OCP, progesterone-only OCP, intrauterine device). Naturally menstruating females experienced significant increases in lean body mass (LBM) and reductions in percent body fat (BF%), though did not improve their isometric strength (~7% vs. ~19%) or 3000 m running time as much as HC users (Myllyaho et al., 2018). Overall, habitual HC use may (Dalgaard et al., 2019; Romance et al., 2019) or may not (Myllyaho et al., 2018) enhance muscle hypertrophic adaptations to resistance training, and HC use seemingly does not affect changes in muscle function, though further corroborating studies are needed.

The current literature demonstrates that the use of HCs has little effect on exercise performance but may worsen the decline in muscle function and cell membrane integrity associated with EIMD. Moreover, HCs seem to blunt acute muscle anabolic responses to exercise, although this does not appear to dampen chronic adaptations to training. However, body composition responses to exercise and HC use can be affected by menstrual dysfunction, which highlights the importance of assessing menstrual status. Overall, present research is limited by its low quality and focus on the OCP with lack of attention to other forms of HCs that may alter responses to exercise, and these should be investigated. Until the role of HCs in acute and

chronic exercise responses is solidified, it is pragmatic to obtain information on HC status and account for HC cycle phase as minimum research standards of practice.

2.5.0. Protein Metabolism and Dietary Protein

Proteins are essential compounds that perform crucial roles in the human body, which include acting as enzymes, hormones, antibodies, and structural components of cells. Proteins, therefore, are vital to overall health. Proteins are obtained through the diet in sources such as meat, fish, eggs, and dairy (animal sources) as well as nuts, seeds, soy, and legumes (plant sources). During the process of digestion, dietary proteins are broken down into their constituent amino acids and, once absorbed and transported to various cells throughout the body, are ultimately used to synthesise new proteins. Importantly, amino acids are involved in the synthesis of skeletal muscle proteins and, by this means, aid muscle repair and regeneration following exercise-induced muscle damage.

2.5.1. Skeletal Muscle Protein Metabolism

Skeletal muscle is a highly plastic tissue that undergoes constant remodelling and repair. The constituent proteins of muscle tissue are continuously synthesised and broken down, termed muscle protein turnover, which occurs at a rate of ~1–2% per day (Welle et al., 1994) with no apparent differences between sexes (Rosa-Caldwell & Greene, 2019). However, the hourly variation in muscle protein turnover rates ranges from 0.14–0.4% depending on activity status and feeding status, i.e. fasted, postprandial, or post-absorptive. The daily net balance of muscle protein is determined by the difference between rates of MPS and muscle protein breakdown (MPB). If MPS exceeds MPB, muscle protein net balance will be positive, resulting in the accretion of muscle protein and, if sustained, skeletal muscle hypertrophy. Conversely, if MPB outweighs MPS, muscle protein net balance becomes negative and results in skeletal muscle atrophy over prolonged periods (Tipton & Wolfe, 2001). Therefore, to maintain muscle protein net balance, and by extension muscle mass, MPS must be stimulated sufficiently and frequently.

The metabolic process of MPS is complex, though it can be divided into two primary processes, namely transcription and translation. Briefly, transcription describes the formation of a new mRNA molecule within the cell nucleus that contains the complimentary base pairs to the DNA of the target protein. This information is then translated to the corresponding amino acids and

peptide bonds bridge the formation of a polypeptide – the primary structure of the newly synthesised protein. These processes are tightly regulated by intracellular proteins, with those involved at the stage of translation initiation being paramount to protein synthesis. The mechanistic target of rapamycin complex 1 (mTORC1), an intracellular protein, serves as the locus of control for translation initiation and is readily activated by nutrition and exercise stimuli. Essential amino acids provide a robust stimulus for mTORC1 (Drummond & Rasmussen, 2008) and without sufficient amino acid availability, protein synthesis is inhibited (Wolfson & Sabatini, 2017). Leucine in particular is a potent stimulator of mTORC1-mediated MPS in males (Atherton et al., 2017; Dreyer et al., 2008; Rowlands et al., 2015) and females (Bukhari et al., 2015; Wilkinson et al., 2018) at rest and following exercise. Therefore, maintaining a high availability of circulating amino acids, notably leucine, via sufficient and regular dietary protein intake is imperative for MPS, net protein balance, and muscle mass maintenance. The current recommendations for the type, amount, and frequency of dietary protein required to optimally stimulate MPS are discussed later in this section.

Muscle protein synthesis also may be stimulated by resistance exercise. Resistance exercise offers a strong stimulus for mTORC1 activation (Drummond et al., 2009; Koopman et al., 2007; Moore et al., 2011) though can also stimulate MPS through the activation of mitogen-activated protein kinases (MAPK) (Moore et al., 2011; Williamson et al., 2003), which regulate the initiation of mRNA translation (Waskiewicz et al., 1999) and play key roles in protein synthesis. The exercise-induced increase in MPS can be measured within an hour following an acute bout of resistance exercise (Dreyer et al., 2008; Dreyer et al., 2006) and is sustained for up to 24 h in trained individuals (MacDougall et al., 1995) and up to 48 h in untrained individuals (Phillips et al., 1997) with no apparent sex differences (Dreyer et al., 2010; Miller et al., 2006; West et al., 2012). Concomitantly, MPB is stimulated during recovery from acute resistance exercise, although to a lesser extent than MPS (Biolo et al., 1995). Nevertheless, without sufficient peri-exercise amino acid provision, muscle protein net balance will remain negative (Biolo et al., 1995; Dreyer et al., 2008; Phillips et al., 1997; Pitkanen et al., 2003). Protein consumption during exercise recovery attenuates MPB and increases MPS rates, resulting in the accretion of muscle protein (Tipton et al., 1999). Accordingly, it was demonstrated in the meta-analysis (n = 49 studies) by Morton et al. (2018) that protein supplementation enhances resistance training-induced gains in FFM, muscle fibre cross-sectional area, and dynamic strength. As such, the combination of resistance training and dietary protein ingestion provides optimal conditions for skeletal muscle adaptation.

2.5.2. Dietary Protein Recommendations – Dose, Timing, and Type

The current recommended dietary allowance stipulates 0.8 $g \bullet d^{-1} \bullet kgBM^{-1}$ of protein be consumed to maintain muscle protein daily net balance, with increased requirements (1.6–2.2 $g \bullet d^{-1} \bullet kgBM^{-1}$) for highly active or athletic populations (Morton et al., 2018; Witard et al., 2019) or during physiological perturbation, such as energy deficiency (Hector & Phillips, 2018) or muscle injury recovery (Tipton, 2015). Where possible, protein recommendations should be met through a food first approach, whereby an individuals' dietary intake majorly comes from whole foods and less so from isolated food components or supplements. The food first approach, while not always appropriate (Close et al., 2022), aids the achievement of other daily nutrient targets, including micronutrients and fibre, and reduces safety risks linked to supplement contamination and adverse effects (Maughan et al., 2018). The current protein intake recommendations for exercising individuals (*Table 2.2.*) stem from research on the acute MPS and chronic muscle responses to various protein feeding protocols, which have manipulated the type, dose, frequency, and timing of protein intake.

Protein Dose

A dose-response relationship between protein intake and MPS stimulation following resistance exercise was demonstrated in trained males by Moore and colleagues (2009). These authors showed that the anabolic response to a post-exercise dose of egg protein was greater with 20 g of protein relative to 0 g, but indifferent between 20 and 40 g doses. The ingestion of 40 g of protein was associated with increased leucine oxidation, which suggests that consuming >20 g of protein post-exercise does not confer additional benefits to MPS rates. Corroborating data by Witard et al. (2014) failed to show an anabolic advantage of ingesting 40 g of whey protein isolate relative to 20 g following resistance exercise in trained males. Again, the 40 g protein dose induced greater oxidative amino acid catabolism, supporting a threshold in the muscle protein synthetic machinery. However, a later study reported a ~20% higher fractional synthesis rate of myofibrillar proteins when double the amount of whey protein (40 vs 20 g) was consumed post-exercise by trained males (Macnaughton et al., 2016). The key difference between the works of Moore, Witard, and Macnaughton is the former two studies utilised a leg-based resistance exercise protocol, whereas the latter study incorporated whole-body resistance exercise. These data suggest a relationship between the number of muscle groups engaged during exercise and the amount of protein required to support muscle anabolism. Substantiating data comes from Holwerda et al. (2019) in which older males performed a single

bout of whole-body resistance exercise then ingested either 0, 15, 30, or 45 g of intrinsically labelled milk protein. Higher rates of myofibrillar protein synthesis were stimulated following the ingestion of the larger protein doses (30 and 45 g) relative to 0 g, and whole-body muscle protein positive net balance occurred in a dose-dependent manner. A dose-response relationship of protein ingestion and MPS rates following resistance exercise has not yet been investigated in females. Due to the various sex differences in skeletal musculature, body composition, and responses to exercise (see section *2.4.0.*), females may have different post-exercise protein requirements to males; albeit an individuals' LBM seemingly does not impact the MPS response to protein and exercise stimuli (Macnaughton et al., 2016). To summarise, studies to date have indicated that, in males, varied doses (20–45 g) of dietary protein are required post-exercise depending on the type of resistance exercise performed, i.e. whole-body or isolated muscle groups, with future study required in females.

Protein Timing

The optimal timing of protein ingestion to maximise exercise adaptations is currently not well defined. The landmark study by Areta and colleagues (2013) used stable isotope tracer methodology to highlight the importance of daily protein distribution. Here, 80 g of whey protein isolate was ingested by young, resistance trained males in a bolus (2 × 40 g), intermediate (4 × 20 g), or pulse (8 × 10 g) pattern over a 12 h post-exercise period. The postprandial plasma aminoacidemia was highest following the ingestion of the bolus protein dose, though only sustained (12 h) in the pulse feeding condition. Nevertheless, the intermediate feeding pattern elicited the greatest increase in myofibrillar fractional synthesis rate during resistance exercise recovery. So, to enhance daily rates of MPS in exercising males, the consumption of ~20 g doses of high-quality protein at regular intervals (~every 3 h) throughout the day is recommended. Research of this kind is yet to be undertaken in females; however, protein feeding pattern did not impact whole-body protein turnover in non-exercising females consuming a high protein (1.7 $g \bullet d^{-1} \bullet kgFFM^{-1}$) diet. Conversely, postmenopausal females benefitted from consuming ~60% of daily protein intake at one meal rather than spread throughout the day (Arnal et al., 1999; 2000), indicating an age-related response. Examination of protein metabolic responses to dietary protein distribution in exercising females would help inform sex-specific recommendations for daily protein intake timing.

There has been a greater wealth of research focussed on peri-exercise, as opposed to daily, protein intake timing. Protein may be consumed before (Tipton et al., 2001), during (Beelen et

al., 2008), or after resistance exercise to support MPS and initiate muscle repair processes. Nonetheless, it is agreed that the optimal anabolic environment for muscle adaptation is provided when protein/amino acids are consumed closely after resistance exercise (Phillips & van Loon, 2011), which may be particularly important for older adults (Esmarck et al., 2001). The timing of protein feeding may also be relevant for chronic training adaptations, although studies are limited. For instance, during a 10-week resistance training programme, male bodybuilders were provided a creatine-enriched protein-carbohydrate supplement either pre- and post- exercise or pre- and post- sleep. Those who received the supplement peri-exercise experienced significantly larger gains in LBM, reductions in BF%, and increases in both squat and bench press maximal strength (Cribb & Hayes, 2006). In contrast, a mixed-protein blend provided to trained males in the same time pattern resulted in comparable between-group adaptations for body composition and strength (Hoffman et al., 2009). These differences could be linked to the anabolic properties of creatine monohydrate (Brose et al., 2003; Kaviani et al., 2019), although the co-ingestion of protein and carbohydrate (Cribb & Hayes, 2006) closer mimics a real-life mixed-meal. The impact of ingesting protein peri-exercise relative to other times of day on exercise adaptations is not understood in females at present.

The importance of protein intake outside of the peri-exercise period, such as pre-sleep to stimulate overnight muscle reconditioning, should not be ignored (Trommelen & van Loon, 2016). It was stressed by Beelen et al. (2008) that ingesting protein exclusively during or immediately after an exercise bout performed in the evening is not sufficient to augment rates of MPS during overnight sleep. Instead, protein consumption immediately before sleep may be necessary to sustain elevated rates of MPS (Res et al., 2012; Trommelen et al., 2018), which can be augmented when prior resistance exercise is performed (Holwerda et al., 2016; Trommelen et al., 2016). Accordingly, it was proposed by Trommelen and van Loon (2016) that protein should be consumed before sleep in addition to at each daily meal to increase total daily protein intake and ensure frequent stimulation of MPS. The optimal dose of protein required to stimulate overnight MPS is not yet established, though a dose-response is apparent. Twenty grams of casein protein with or without additional (1.5 g) leucine consumed by older males failed to increase myofibrillar fractional synthesis rate during overnight sleep relative to placebo. Twice the casein dose (40 g) was required to induce overnight protein anabolism (Kouw et al., 2017). Likewise, 40 g of casein protein was sufficient to increase overnight MPS rates (Res et al., 2012) but 30 g was not (Trommelen et al., 2018) when consumed by younger males. The higher pre-sleep protein requirements may be due to the prolonged time spent in a

fasted state during sleep relative to in between meals during waking hours (i.e. 8 vs 4–5 h). Nevertheless, it is unknown whether chronic pre-sleep protein ingestion translates to superior muscular adaptations relative to when protein is consumed at other times of day. As such, acute and longitudinal examination of muscle responses to incremental protein doses ranging 20–40 g from a variety of protein sources is required in both males and females to establish protein recommendations for optimal overnight muscle reconditioning.

Protein Type

The amino acids required to support skeletal muscle reconditioning may be derived both from animal and plant sources of dietary protein. Animal-based protein sources are deemed to possess superior anabolic properties than plant-based protein sources and indeed, have shown to stimulate higher rates of MPS at rest and following resistance exercise (Gorissen et al., 2016; Tang et al., 2009; Wilkinson et al., 2007), though this is not always the case (Pinckaers et al., 2021). The superior anabolic potential of animal-based relative to plant-based proteins has been attributed to their greater bioavailability and higher essential amino acid content, particularly leucine (Gorissen et al., 2018; van Vliet et al., 2015). Due to their lower quality, a greater amount and/or variety of plant-based proteins at each feeding occasion may be required to stimulate rates of MPS that are comparable to those measured following the ingestion of animal-based proteins (Gorissen et al., 2016; Kouw et al., 2021). For instance, a 20-g protein dose can be obtained through the consumption of 200 g of dairy yoghurt relative to ~500 g of soya yoghurt, which may be impractical or cause gastrointestinal discomfort, especially close to exercise or pre-sleep. Therefore, individuals striving to meet daily protein recommendations through plant-based sources alone may benefit from supplemental sources of protein (e.g. isolated pea, soy, rice, hemp, or blended protein) in line with the ‘food first but not always food only’ approach (Close et al., 2022).

Protein sources differ in their rate of digestion and absorption, which too impacts the postprandial MPS response. Quickly digestible proteins, such as whey, result in rapid but brief postprandial plasma aminoacidemia, contrasted by the moderate but prolonged increase in circulating amino acids when slowly digestible proteins, such as casein, are consumed (Pennings et al., 2011). By this means, the ingestion of whey compared to casein protein augments MPS rates in the early (<6 h) postprandial period both at rest and following resistance exercise (Burd et al., 2012; Pennings et al., 2011; Tang et al., 2009). Whereas to raise MPS rates for sustained periods (~8 h e.g. during overnight sleep), casein is the preferred option,

though higher doses (~40 g) may be required (Kouw et al., 2017; Res et al., 2012; Trommelen et al., 2018). Milk protein is a source of casein (80%) and whey (20%) and hence provides a mixture of slowly and quickly digestible proteins. As such, several studies have investigated whether milk protein ingestion can stimulate both rapid and sustained increases in MPS above basal. For instance, resting MPS rates remained elevated by 86% 5 h after the consumption of 38 g of milk protein concentrate by young males (Van Vliet et al., 2019). Whereas the ingestion of 20 g of milk protein concentrate by middle-aged males increased myofibrillar fractional synthesis rate relative to basal for only 90 min with no superiority over the ingestion of 20 g whey protein concentrate (Mitchell et al., 2015). Such disagreement might be due to the lower protein dose or to an age-related decline in the anabolic response to amino acid provision (Breen & Phillips, 2011). When the consumption of skimmed milk providing 18 g of protein was preceded by a high-intensity bout of leg-based resistance exercise, mixed-muscle fractional synthesis rate was elevated to a greater (34%) extent than when an isonitrogenous and isoenergetic dose of soy protein was consumed by young, trained males. Further, milk compared to soy protein ingestion better sustained positive net protein balance (Wilkinson et al., 2007), which is needed for the accretion of protein within skeletal muscle. Milk protein also has shown to be anabolically superior to carbohydrate ingestion following submaximal running in trained males (Lunn et al., 2012) and comparable to whey and micellar casein protein after concurrent exercise in recreationally active males (Churchward-Venne et al., 2019). Therefore, milk protein can be considered an ideal protein source to be consumed at any time of day, at rest or following multiple modes of exercise, to stimulate prolonged muscle protein anabolism in male populations. The impact of milk protein ingestion on resting and post-exercise MPS has not yet been examined in female populations.

Frequent and sufficient stimulation of MPS culminates in skeletal muscle fibre hypertrophy and subsequently, an increase in total muscle mass and strength. The impact of chronic milk protein ingestion on muscle adaptation has been considered in exercise training interventions. The review by Phillips and colleagues (2009) identified that, across 9 studies (92.5% male sample), the performance of 8–16 weeks of resistance training concurrent with the consumption of milk protein induced similar increases in LBM compared with whey protein (2.7 vs 2.9 kg) but greater increases than soy (1.4 kg) or carbohydrate (0.9 kg). Since this review, others have provided support for the regular consumption of concentrated milk protein (30 g) (Pourabbas et al., 2021) and fat-free Greek yoghurt (20 g) (Bridge et al., 2019) after resistance exercise and pre-sleep for LBM and dynamic strength adaptations in young males, while others disagree

(Rankin et al., 2004). Generally, these outcomes have demonstrated that the acute stimulation of post-exercise MPS with milk protein (Churchward-Venne et al., 2019; Lunn et al., 2012; Wilkinson et al., 2007) translates to chronic exercise adaptations with frequent milk protein ingestion. Notwithstanding, the representation of females in this area of research has not greatly improved over the past decade. For instance, 12 weeks of whole-body heavy (80% 1RM) resistance training was completed by young, recreationally active females in the study by Josse et al. (2009). Immediately and 1 h post-exercise, 18 g of protein in the form of skimmed milk or an isoenergetic carbohydrate drink (0 g protein) was consumed. Greater increases in FFM and reductions in fat mass were measured using DXA in the females who consumed milk. Gains in dynamic strength for multiple exercises were comparable between supplement groups, which is in line with previous observations from the study implemented in trained males by the same authors (Hartman et al., 2007). It was later suggested by Josse and colleagues (2011) that milk protein can enhance body composition during dietary- and exercise- induced weight loss. For 16 weeks, aerobic and resistance training was performed by overweight/obese sedentary females alongside 500 kcal•d^{-1} energy restriction. The diet comprised either 0.7, 0.8, or 1.3 g•d^{-1}•kg^{-1} of protein with 1, 3–4, or 6–7 daily portions of dairy, respectively. Females who consumed the most total protein and servings of dairy lost the greatest amount of fat mass and BF%, while LBM was increased. However, even with an adequate protein intake (0.8 g•d^{-1}•kg^{-1}), increased dairy consumption preserved LBM during total body mass reduction relative to the low dairy diet. Therefore, it may not be the case that higher protein intakes are required during energy deficiency, as currently recommended (Hector & Phillips, 2018), with instead the source of protein being of importance for muscle mass retention and skeletal muscle health.

To summarise, current evidence suggests that individuals require 20–40-g doses of high-quality protein at regular intervals throughout the day, with particular attention paid to the post-exercise and pre-sleep period, to support skeletal muscle remodelling and adaptation. The optimal protein dose may depend on the type of exercise performed, age, training status, and possibly sex of the individual, and the time of day. A moderate dose (~20 g) of quickly digestible protein (e.g. whey) is perhaps ideal when the period between feeding occasions is brief (4–5 h), while a larger dose (~40 g) of slowly digestible protein (e.g. casein) is preferential when the postprandial period is prolonged (>6 h). Convenience and feasibility likely impact an individuals' choices around protein intake. Occasionally, supplemental protein sources are preferred, for instance to avoid large quantities of food consumption or excessive energy intake, but where possible, protein should be predominantly obtained from whole-food sources. Future

studies should confirm whether these protein intake recommendations are appropriate for females via acute and longitudinal examination of female muscle responses to incremental protein doses from multiple sources.

Table 2.2. A summary of protein intake recommendations for exercising individuals

	Recommendation
Type	• High-quality animal-based protein (e.g. milk, beef, egg) or plant-based protein (e.g. soy, pea, wheat) • Predominantly from whole-food sources • Blending or enriching plant-based proteins can increase their anabolic potential
Dose	• 20–40 g • A higher amount (~40 g) of single-source plant-based protein is likely required, although a dose-response study has not been conducted
Timing	• Every 3–4 h throughout the day • Peri-exercise (post-exercise is optimal) • Pre-sleep (preferably a slowly-digestible protein source [e.g. casein]; a higher dose [~40 g] may be required)

Data from: Areta et al. (2013); Gorissen et al. (2016); Holwerda et al. (2019); Kouw et al. (2017, 2021); Macnaughton et al. (2016); Moore et al. (2009); Phillips & van Loon (2011); Trommelen & van Loon (2016); Witard et al. (2014).

2.6.0. Resistance Exercise

Resistance exercise is a form of activity that causes muscular contraction against an external resistance. Alone, or in combination with endurance-type exercise, resistance exercise presents many health benefits not limited to: improving cardiovascular function (Fleck, 1988), aiding the management of type II diabetes (Eriksson et al., 1998), improving dynamic stability (Anderson & Behm, 2005), reducing the risk of osteoporosis and sarcopenia (Beckwée et al., 2019; Layne & Nelson, 1999), modifying body composition (Schoenfeld, 2010), and enhancing psychological wellbeing (Kekäläinen et al., 2018). There are several intrinsic and extrinsic variables that may influence both acute and chronic responses to resistance exercise, and these can be manipulated to maximise exercise adaptations.

2.6.1. Extrinsic Variables and Training Adaptations

The performance of repeated bouts of resistance exercise culminates in training adaptations, i.e. increases in muscle mass and strength (Schoenfeld, 2010). Individuals unaccustomed to resistance exercise can experience rapid adaptations, which typically plateau after several months of training. Therefore, gradual increases in the physiological demands placed upon the body during exercise – known as progressive overload – are required to ensure continual improvement. Accordingly, several extrinsic variables of the exercise may be manipulated to achieve progressive overload, being the absolute or relative intensity (load) of the movement; the repetition tempo; the period of rest between sets and movements; the repetition number within each set; and the total work volume (calculated as repetitions × sets × load). The severity of adjustment of these variables is dependent upon the training experience and goals of the individual. For instance, untrained individuals are recommended to utilise lighter loads, longer rest periods, and lower training frequencies relative to experienced individuals. Current resistance training guidelines are broadly divided into enhancing muscle strength and hypertrophy, though may be tailored to increasing muscle power, local muscle endurance, and motor performance (ACSM, 2009). A summary of the strength- and hypertrophy- based training guidelines classified by training experience can be found in *Table 2.3.*

Table 2.3. A summary of resistance training guidelines for strength and hypertrophy adaptations

Training type	Training status	Load (% 1RM)	Repetition number	Set number	Rest period (min)	Concentric movement velocity	Exercise frequency ($d \bullet wk^{-1}$)
Strength	Untrained	60–70%	1–6	1–3	2–3	Slow-moderate	2–4
	Trained	80–100%	1–6	1–3	2–3	Slow-fast	4–6
Hypertrophy	Untrained	70–85%	6–12	1–3	1–2	Slow-moderate	2–4
	Trained	70–100%	6–12	3–6	2–3	Slow-fast	4–6

Data from the American College of Sports Medicine and the National Strength and Conditioning Association (ACSM, 2009; Sands et al., 2012).

Acute responses to the manipulation of extrinsic resistance exercise variables may be quantified through the measurement of muscle protein turnover rates and EIMD, while chronic responses can be interpretated by changes in muscle mass and strength. In particular, these responses have been extensively studied following manipulation of resistance exercise load. As discussed in detail in *chapter 5*, varying the load of resistance exercise is an effective management strategy for EIMD, with lower exercise loads inducing less muscle damage, while promoting comparable acute and chronic adaptations to exercise performed with higher loads. The current training recommendations pertaining to exercise load are based on the notion that

full motor-unit recruitment is required to stimulate increased rates of post-exercise MPS and, by extension, training-induced muscle hypertrophy. The 'size principle' would stipulate that low-load resistance exercise is capable of activating only the slower and lower force-producing motor units, while failing to activate the faster, higher force-producing units (Henneman et al., 1965). However, this ideology has been challenged by Burd and colleagues (2010), who demonstrated that low-load resistance exercise (30% 1RM), when performed to volitional failure, resulted in maximal motor unit recruitment. Subsequently, MPS rates were elevated to a similar extent in the early (4 h post-exercise) recovery period and better sustained into the late (21–24 h) recovery period following low-load relative to high-load (90% 1RM) resistance exercise. The anabolic potential of low-load resistance exercise may be attributed to the high total volume of work performed (Burd et al., 2010). Whether the transient elevation in MPS rates stimulated by low-load resistance exercise translates into chronic adaptations of comparable magnitude to high-load resistance exercise is still debated.

The most comprehensive review of training adaptations from resistance exercise of varied load was presented by Schoenfeld and colleagues (2017_a). The accompanying meta-analysis (n = 21 studies) revealed that training-induced adaptations significantly favoured high-load for 1RM (ES: high-load = 1.69 ± 0.23 vs. low-load = 1.32 ± 0.23; p = 0.003) but not for maximal isometric strength nor muscle hypertrophy. Although there were insufficient studies to meta-analyse data for the change in LBM, no significant differences between high-load (6–12 RM) and low-load (20–30 RM) exercise conditions were demonstrated for young, untrained males (Au et al., 2017; Morton et al., 2016; Tanimoto et al., 2008) or females (Rana et al., 2008; Schuenke et al., 2012) at an individual study level. While it was suggested by this review that training load is not a determinant of hypertrophic nor isometric strength gains, it should be noted that studies in which the resistance exercise was performed to volitional failure were exclusively included by Schoenfeld et al. (2017_a). Thereby, in the majority (81%) of studies included, a greater total work volume was performed within the low-load conditions. In the few studies that utilised volume-equated exercise protocols, significantly greater increases in bench press (Anderson & Kearney, 1982) and leg-based (Campos et al., 2002) 1RM with high-load resistance training compared to low-load were experienced by young, untrained males. Whereas increases in upper- and lower- body maximal strength occurred independently to the exercise load in young, untrained females (Hisaeda et al., 1996; Stone & Coulter, 1994). These data support the notion that exercise volume is not a prerequisite to training-induced strength

adaptations (Barcelos et al., 2015; Schoenfeld et al., 2019), though perhaps suggests a sex-specific response (discussed in section *2.6.2.*).

Exercise volume may be a stronger determinant of muscle hypertrophy than muscle strength. However, a large degree of variability within studies is evident (Schoenfeld et al., 2017_b) and only a couple of studies have demonstrated a clear beneficial effect on muscle hypertrophy with higher exercise volumes (Correa et al., 2015; Radaelli et al., 2015). Likewise, the duration of inter-set rest cannot be confirmed to have bearing on muscle hypertrophic adaptations due to a paucity of studies and unclear direction of the findings (Grgic et al., 2017). It seems plausible to manipulate the duration of the inter-set rest period depending on the intensity and type of exercise, as opposed to with intent of maximising adaptations. Accordingly, near-maximal (Wernbom et al., 2007) and multi-joint free weight (Senna et al., 2016) exercise movements likely necessitate longer inter-set rest periods.

In summary, the extent by which the manipulation of resistance exercise extrinsic variables impacts acute and chronic responses to training is confounding. Manipulating the relative exercise load may influence maximal isotonic strength, with preference towards higher loads, but does not appear to influence maximal isometric strength, muscle hypertrophy, whole-body lean mass change, nor MPS rates. The degree to which training adaptations are determined by the total work volume and inter-set rest period of the exercise is unclear at present, largely owing to heterogeneity between the few available studies. Further, much of the current data has been obtained from young, untrained male populations and adaptations to resistance training are likely influenced by age, sex, training status, and other intrinsic characteristics. As such, future research and training recommendations should consider both extrinsic and intrinsic variables to optimise resistance training adaptations.

2.6.2. Intrinsic Variables and Training Adaptations

Resistance training adaptations may, in part, be predetermined by intrinsic variables including, but likely not limited to an individual's age; sex; hormone milieu (see section *2.4.0.*); and fibre-type distribution and size (see section *2.2.2.*). Males and females may experience divergent responses to resistance training, stemming from sex differences in skeletal musculature, hormone profile, baseline strength and body composition, and early-stage neural effects. A quantitative summary of sex differences in hypertrophic and strength adaptations to resistance training was provided in the systematic review with meta-analysis by Roberts et al. (2020). Of

the 50 total studies included, no significant differences existed between males and females for resistance training-induced relative muscle hypertrophy (n = 10 studies) nor relative lower-body strength gains (n = 23 studies). Conversely, the change in relative upper-body strength significantly favoured females (mean between-sex ES = −0.60 ± 0.16; 95% CI [−0.93, −0.26]; p = 0.002; n = 17 studies). One study in particular, in which 20 weeks of unilateral elbow flexor exercise was performed by participants, reported relative strength increases among females that were ~2.5-fold those measured among males (O'hagan et al., 1995). However, even upon omission of this study and other potentially influential studies (Carlsson et al., 2017; Hostler et al., 2001) from the analysis, females still had superior upper-body strength gains compared with their male counterparts. The reason for females experiencing superior upper-body, but comparable lower-body, strength gains to males is unclear, though may relate to the habitual activities of males, i.e. occupations involving heavy lifting. Due to the potential sex differences in resistance training adaptations, it is important to study or analyse data for males and females separately during training intervention studies and resistance training guidelines should be sex-specific.

Differences between populations regarding long-term muscle adaptations to resistance training could originate from early-stage neurological adaptations. During the early stages of resistance training (<5 weeks), neurological adaptations drive the initial increase in muscle strength, resulting in disproportionate strength gain relative to muscle mass gain (Moritani, 1979). Evidence in support of neural adaptations has been provided by electromyostimulation and cross-over training research, whereby increases in strength have occurred in the absence of voluntary muscle contraction-induced morphological changes, e.g. myofibre growth, limb enlargement, and altered muscle enzyme activity (Zhou, 2000). These initial neural effects of training can increase motor unit synchronisation, improve coordination, and aid movement learning. So, even simple actions require a degree of skill and practice for optimal movement and, by extension, maximal strength to be achieved (Folland & Williams, 2007). Accordingly, untrained individuals who are more susceptible to neurological adaptations are unlikely to achieve their true maximal strength during primary assessments of MVC. Repetition of the same task will likely result in enhanced performance after a degree of muscle learning has occurred (Folland & Williams, 2007). Therefore, conducting a second MVC test to confirm maximal strength prior to experimental intervention is good practice. Further, there is evidence to suggest that neurological adaptations are influenced by sex, meaning that males and females may experience disparate rates of strength development. It was suggested by Delmonico et al.

(2005) and Ivey et al. (2000) that males are more dependent on morphological adaptation, i.e. muscle hypertrophy as a prerequisite to strength adaptations, whereas in females, non-morphological adaptations are a stronger influence on resistance training-induced strength gains, suggesting that females experience neural effects more than males. Lending support to this notion, it was shown by Rutherford and Jones (1986) that during the first 6 weeks of resistance training, females experienced greater improvement in training load relative to males. These sex differences could be linked to the greater muscle activation during contraction in females compared with males (Bolgla et al., 2014; Dwyer et al., 2010; Zeller et al., 2003). Muscle activation data are used to prescribe exercises that target isolated muscles and therefore, these studies suggest that sex-specific exercises may be beneficial to maximise muscle activation and optimise adaptations.

2.7.0. Conclusion

Herein, an overview of skeletal muscle has been provided, encompassing its structure and mechanisms of contraction, damage, and repair, which may be influenced by several factors, not limited to sex, hormonal status, training status, and body composition. The degree of influence of these variables is difficult to ascertain, as most current studies are limited by their poor consideration for methodological design, as well as the inherent disadvantages of using indirect markers of muscle damage. Further, this work identified disparate findings between EIMD studies conducted with animals and humans, partly due to the difficulties with controlling all variables in humans, particularly when attempting to isolate the impact of sex hormones. This discord adds to the challenge of solidifying the mechanisms of EIMD and symptom development. Nonetheless, the relevance of acute EIMD for chronic exercise adaptations is undetermined and warrants further investigation in males and, in particular, females.

Accordingly, this chapter has also highlighted a need for further sex comparative and female-focussed research, owing to many physiological sex differences that may impact acute and chronic responses to resistance exercise and protein feeding. While in recent years more female-focussed research has emerged, studies have produced equivocal findings, which seems due in large to methodological inconsistencies; lack of control over female-specific variables, e.g. hormonal status; and a limited number of high-quality studies conducted with females or both sexes. Moving forward, uniformity in study design and agreement on the best-practice procedures for conducting research with females, as is beginning to form (Elliott-Sale et al.,

2021), will help confirm the role of sex in responses to nutrition and exercise stimuli. Doing so would enable practical guidelines for exercise and nutrition to be tailored accordingly to males and females.

CHAPTER 3 The impact of dietary protein supplementation on recovery from resistance exercise-induced muscle damage: A systematic review with meta-analysis

3.1.0. Prelude

This PhD work has centred around muscle recovery strategies following resistance exercise, and dietary protein supplementation is a chosen recovery strategy by many exercisers. Therefore, it was considered important to scope what research had already been conducted surrounding dietary protein in the context of EIMD and identify where knowledge gaps lay in order to design an appropriate, novel study. This review was intentionally exclusive to studies that incorporated resistance exercise as the muscle damage-inducing exercise mode to fit with the wider theme of this thesis; although, it is appreciated that the method used to induce muscle damage may be deemed unimportant when establishing EIMD management strategies.

This chapter has been published in the European Journal of Clinical Nutrition.

3.2.0. Introduction

The World Health Organisation physical activity guidelines stipulate adults should perform resistance exercise at least twice per week to benefit general health, quality of life, and healthy weight maintenance (Bull et al., 2020). Resistance training can elicit improved skeletal muscle mass, strength, stability, glucose tolerance, and bone density (Anderson & Behm, 2005; Eriksson et al., 1998; Layne & Nelson, 1999; Schoenfeld, 2010; Tan, 1999). Nevertheless, unaccustomed resistance exercise, particularly involving eccentric contractions, can damage skeletal muscle fibres (Stauber, 1989) mediated by the combined disruption to both sarcomeres and the excitation-contraction coupling system (Proske & Morgan, 2001_a; Warren et al., 1993). Resistance EIMD presents physiological and mechanical consequences that may delay exercise recovery and limit future exercise quality, owing to reduced muscle function. For example, EIMD can induce muscle soreness and swelling and reduce muscle force generating capacity by 50% (Hesselink et al., 1996; Nosaka et al., 2002_b; Nosaka & Newton, 2002), which may persist for seven days post-exercise (Byrne & Eston, 2002; Farup et al., 2014). Subsequently, acute EIMD can dampen chronic adaptations to resistance training (Damas et al., 2016_b). Successive exposures to comparable exercise stimuli attenuate EIMD owing to the RBE (Fridén et al., 1983; Hough, 1902; Schwane & Armstrong, 1983). However, even mild symptoms of muscle soreness and weakness could diminish personal motivation to exercise and reduce the frequency and/or quality of exercise sessions. Furthermore, in line with resistance training guidelines, individuals frequently alter exercise load, repetition range, or volume, thus imposing new exercise stimuli and susceptibility to EIMD. Therefore, EIMD may obstruct the benefits of regular (≥twice weekly) and progressive resistance exercise.

The severity of EIMD can be assessed directly (i.e. through muscle biopsy sampling and magnetic resonance imaging) or indirectly (i.e. through tests of muscle function, subjective soreness, and blood analysis of intramuscular proteins). While direct assessments may seem the preferred option, muscle biopsy sampling is invasive and presents two inherent assumptions: that damage is inflicted by the intended intervention and not the biopsy procedure itself; and that the damage measured within the sample reflects that of the whole muscle (Clarkson & Hubal, 2002; Malm et al., 2000). To this end, indirect markers are preferentially employed to indicate EIMD (Warren et al., 1999), with isometric and isokinetic tests of muscle function considered the most valid and reliable (Morton et al., 2005). Other indirect markers of EIMD, including muscle soreness and blood [CK], are limited by their high inter- and intra-

individual variability (Damas et al., 2016$_a$; Nosaka & Clarkson, 1996$_c$) though are frequently assessed in research, allowing for between-study comparisons.

Training adaptations are potentially hindered by EIMD (Damas et al., 2016$_b$) and hence several strategies have been investigated to mitigate EIMD, including cryotherapy, massage, stretching, compression garments, electrostimulation (Dupuy et al., 2018; Torres et al., 2012), and dietary manipulation. Dietary strategies have received considerable recent attention, especially regarding supplemental protein- and amino acid- based products provided peri-exercise (Davies et al., 2018; Fouré & Bendahan, 2017; Harty et al., 2019; Pasiakos et al., 2014; Poulios et al., 2019; Rahimi et al., 2017; Rahimi et al., 2018).

Peri-exercise protein consumption is a common strategy to enhance post-exercise recovery and training adaptation by stimulating increased rates of MPS (Tipton, 2008). Following exercise, MPS is stimulated to repair damaged muscle proteins, and MPS rates are further augmented by peri-exercise protein consumption (Moore et al., 2009). Given that peri-exercise protein consumption also may reduce muscle damage (Davies et al., 2018; Harty et al., 2019; Poulios et al., 2019), it follows that protein supplementation may co-benefit muscle recovery by reducing EIMD and enhancing MPS rates. Accordingly, several sources of protein, including whey, casein, soy, wheat, and milk, have been investigated as nutritional strategies for mitigating EIMD.

Despite extensive research, the evidence for peri-exercise protein supplementation attenuating EIMD remains inconclusive. Following resistance exercise, declines in maximal strength have been attenuated with milk protein ingestion in some (Cockburn et al., 2008; Draganidis et al., 2017), but not all (Cockburn et al., 2010; 2012; Rankin et al., 2015) studies. Recently, regular consumption of whey but not pea protein during 96 h of exercise recovery lowered peak elevations in serum [CK] relative to water ingestion (Nieman et al., 2020). However, whey protein intake failed to ameliorate muscle soreness; consistent with other observations, irrespective of feeding timing (Kim et al., 2017; White et al., 2008). Moreover, the impact of whey protein supplementation on EIMD is apparently influenced by whether hydrolysed or isolated whey protein is provided, despite equivalent dosing protocols (Buckley et al., 2010; Dale et al., 2015). The variety of exercise protocols, protein dosing and timing regimes, participant characteristics, dietary controls, and measurement tools employed among studies likely contribute to the diverse findings. Drawing conclusions on the efficacy of dietary protein for managing EIMD requires a systematic approach with account for methodological design.

The systematic reviewing of relevant literature has failed to produce definitive conclusions, perhaps due to the overly broad or narrow inclusion criteria specified (Davies et al., 2018; Pasiakos et al., 2014). No review to date has explicitly analysed studies whereby a variety of protein supplements were consumed in conjunction with resistance exercise. Studies that utilised varied exercise protocols (resistance and endurance) and provided protein- or amino acid- based supplements were systematically reviewed by Pasiakos and colleagues (2014). Resistance exercise typically causes more severe EIMD than endurance exercise, although only five of the 27 included studies involved resistance exercise alongside protein consumption (2014). Therefore, this review cannot conclude the impact of protein supplementation on resistance EIMD. Conversely, the impact of protein supplementation on EIMD following resistance exercise was assessed in a systematic review (n = 8) by Davies and colleagues (2018). However, the inclusion criteria were limited to studies exclusively examining the response of muscle function to whey protein supplementation, without consideration for other protein sources and EIMD markers. The accompanying meta-analysis revealed an overall small-medium beneficial effect of whey protein in restoring muscle function during exercise recovery. Nonetheless, the impact of a range of protein sources on various EIMD markers is currently unknown. Therefore, the purpose of this systematic review and meta-analysis was to establish what the current literature suggests about the overall impact of peri-exercise protein supplementation on indirect markers of resistance exercise-induced muscle damage. This study sought to achieve this aim by comparing the change from pre-exercise in MVC, muscle soreness, and [CK] at <24, 24, 48, 72, and 96 h following acute resistance exercise between protein and control intervention groups. The outcomes of this review will inform protein intake recommendations specifically for resistance exercise recovery.

3.3.0. Methods

3.3.1. Inclusion Criteria

The analysis was confined to studies published in English-language journals that met the following criteria: (1) an experimental trial that involved acute (single-bout) resistance exercise; (2) included ≥1 indirect measure of muscle damage; (3) muscle damage was measured for ≥24 h post-exercise; (4) included peri-exercise supplementation with protein versus control (including carbohydrate); (5) involved adult participants with no known clinical condition or musculoskeletal injury. Studies were excluded if conducted in participants <18 y, animals, or *in vitro* models, or included another protein supplement as the control, supplements containing

therapeutic or ergogenic aids (e.g. polyphenols, antioxidants), or physiotherapeutic/pharmaceutical methods targeting muscle damage (e.g. massage, cryotherapy). Studies that involved endurance-type exercise, unloaded resistance exercise (e.g. drop-jumps), concurrent exercise (if the dominant exercise was not resistance-type), or chronic resistance training were excluded, as these exercise modes induce varied severities of EIMD. Therefore, it would be inappropriate for this meta-analysis to group different modes of exercise and further, this review aimed to inform sport nutrition guidelines exclusively for resistance exercise.

3.3.2. Search Strategy

The literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009) on PubMed, SPORTDiscus, and Web of Science databases for studies published up to March 2021 using the following syntax: (muscle damage OR muscle injury OR muscle soreness OR exercise recovery OR exercise induced muscle damage OR EIMD) AND (protein OR "milk*" OR whey OR soy OR casein OR wheat OR beef) NOT (running OR cycling OR rats OR mice). Articles were assessed for eligibility by two independent reviewers using the Rayyan software (Ouzzani et al., 2016). The reference lists of eligible articles were also screened.

3.3.3. Coding of Studies and Data Extraction

Studies were read and individually coded by two independent reviewers (AP and LM) for the following variables: (1) author, title, and year of publication: (2) participant demographic (sex, age (categorised as per Schoenfeld et al., 2017$_a$), training status, and sample size); (3) resistance exercise protocol, including exercise mode, load, set and repetition number, and inter-set rest duration; (4) type of protein and control supplement used; (5) total daily protein intake; (6) protein dose and timing protocol; (7) assessment methods of muscle damage; (8) measurement time-points in relation to exercise; and (9) significant findings. For analyses, mean and standard deviation (SD) (absolute or change from baseline), and sample size data were extracted for each variable and time-point for treatment and control groups. Study authors were contacted to provide raw data and if not received, these data were extracted from reported figures using WebPlotDigitizer.

3.3.4. Methodological Quality

Study quality was assessed by two independent reviewers (AP and LM) based on the 11-point PEDro scale, which is considered reliable and valid for quality-assessing randomised controlled trials (Elkins et al., 2010; Maher et al., 2003). Ratings were categorised as: 0–3 = 'poor'; 4–5 = 'fair'; 6–8 'good'; and 9–10 = 'excellent'.

3.3.5. Meta-analyses

Each within-study comparator group (protein vs control) was treated as an independent trial. A meta-analysis was conducted if the total number of trials (*k*) was ≥3 to generate *k* independent ESs. Separate meta-analyses (16 total) were conducted at each post-exercise time-point (<24, 24, 48, 72, and 96 h) for the most measured variables: isometric MVC, isokinetic MVC, serum/plasma [CK], and muscle soreness VAS/VRS score. If multiple measures of these variables were obtained, e.g. leg extension and flexion MVC or active and passive muscle soreness, the mean of these values was used in the analysis.

For each trial, a Hedges' *g* ES with correction for small positive bias was calculated due to dissimilar within-study group sample sizes (Borenstein et al., 2021). For MVC and [CK], ESs were calculated from mean and SD values as a percent change from baseline. For muscle soreness, there were insufficient data to use percent change from baselines values, due to muscle soreness being either not measured or reported as zero at baseline. Therefore, muscle soreness ESs were calculated using the absolute mean and SD at each time-point. Weighted ESs were calculated using the standard error of the effect and adjusted with Tau squared (τ^2).

Trial heterogeneity within each meta-analysis was assessed using Cochran's Q (*Q*) and I^2 (Higgins et al., 2003). Due to the moderate-substantial heterogeneity detected, a random-effect model was used to calculate pooled ESs, which are reported with 95% confidence intervals (CIs) (Borenstein et al., 2021). Effect sizes are interpreted as 0.2 = 'small'; 0.5 = 'medium'; and 0.8 = 'large' with statistical significance determined by zero overlap of the 95% CI range (Cohen, 1992). To identify potentially influential trials, a sensitivity analysis was conducted by performing meta-analyses with removal of each trial one at a time. Trials were considered influential if their removal resulted in the pooled ES changing from significant to non-significant or *vice versa*. Pooled ESs with removal of influential trials are reported in the manuscript text and forest plots display all trials.

The magnitude of EIMD was determined for trials included in the meta-analyses based on the relative peak reduction from baseline in MVC, as per Paulsen et al. (2012): mild = <20%,

moderate = 20–50%, and severe = >50% reduction. For studies providing insufficient data to be meta-analysed (i.e. do not report mean change and variation) or when k <3, the mean percent change values were calculated.

3.4.0. *Results*

3.4.1. Study Quality and Overview

The literature search yielded 586 articles, of which 38 potentially met the inclusion criteria based on abstract screening (*Figure 3.1*). After full-text screening, 29 studies were confirmed to meet the inclusion criteria and were included in the systematic review (*Table 3.1*).

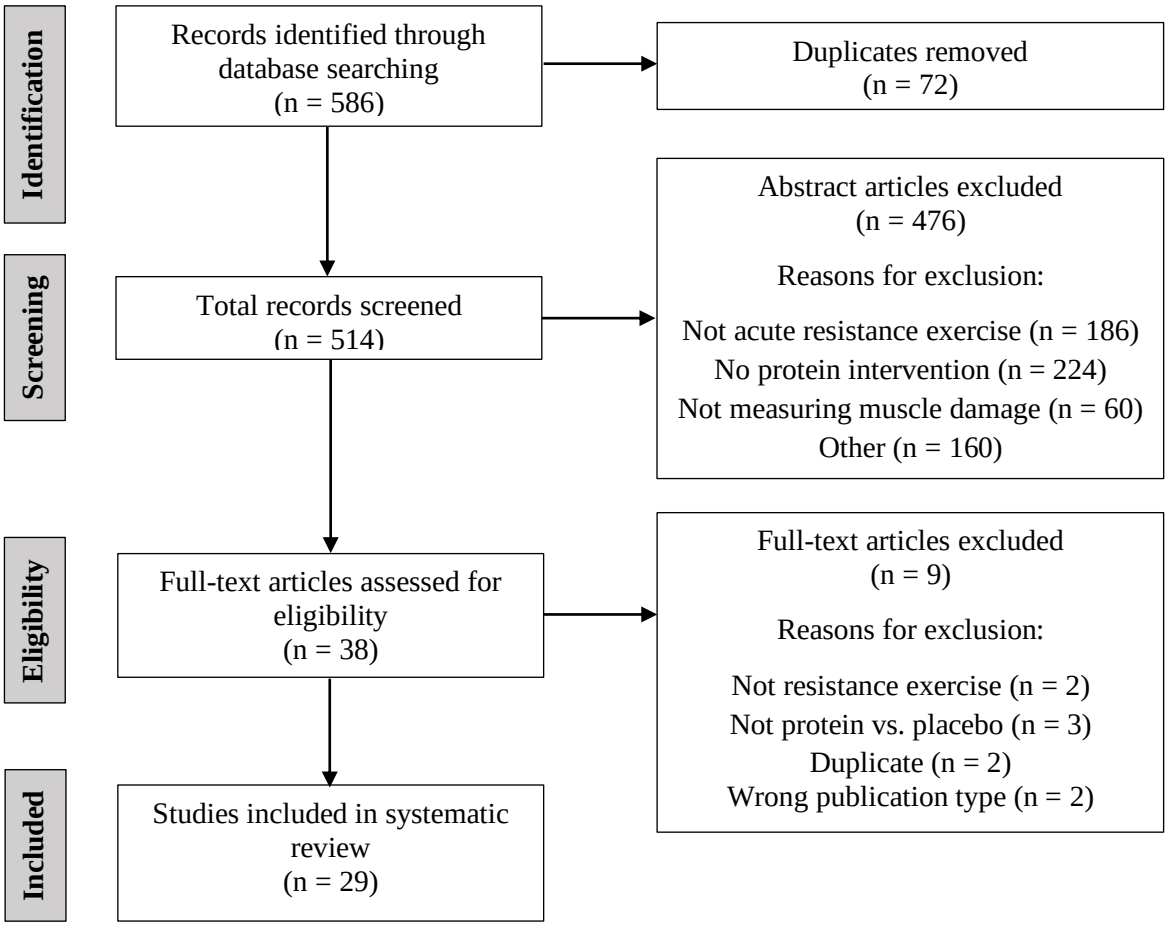


Figure 3.1. Flow diagram of the literature search process.

The 29 studies consisted of 45 trials, of which 26 studies and 40 trials were included in ≥1 meta-analyses. Three studies were not included in any meta-analysis (Baty et al., 2007; Burnley et al., 2010; Samadi et al., 2012) due to insufficient data, and the mean percent change values

are reported in *Table 3.2*. Methodological quality ratings are included in *Table 3.1*. The mean and median rating of study quality were 7 and 8, respectively, indicative of good quality. Only one study was categorised as poor and 13 as excellent.

In total, 763 participants (94% young males) were included. Fourteen studies were conducted with trained individuals and 15 with untrained. The muscle-damaging resistance exercise was restricted to lower-body muscle groups in most studies; upper-body in one study (Kim et al., 2017), and whole-body in nine studies. Muscle contractions were concentric-eccentric (n = 16 studies) or eccentric-only (n = 13 studies). The magnitude of EIMD was predominantly mild or moderate, with only one study reporting severe EIMD (Kim et al., 2017). EIMD magnitude seemingly did not influence the response to protein supplementation (*Supplementary Table S3.1*).

Protein was provided pre-exercise (n = 1) (Hasegawa et al., 2014), post-exercise (n = 16 studies), or pre- and post- exercise (n = 12), including 3 studies that investigated supplementation timing (Cockburn et al., 2010; Kim et al., 2017; White et al., 2008). Whey protein (including hydrolysed and isolated forms) was the most common protein source used, either alone (n = 10 studies) or combined with carbohydrate (n = 10 studies). Eight studies provided milk-based protein, and 4 studies included other protein sources (whey, casein, and collagen blend (Hoffman et al., 2010), pea protein (Nieman et al., 2020), rice and pea protein (Saracino et al., 2020), egg white and soy protein (Hasegawa et al., 2014)). The control group supplements were either carbohydrate-based (n = 9), a non-isoenergetic liquid (e.g. artificially-sweetened water) (n = 11), both (n = 5), or no supplement provided (n = 3). Except for one study (Grubic et al., 2019), all supplements were liquid. Daily protein intake with exclusion of the supplement was adequate in all trials in the protein groups (0.8–2.1 g•kgBM^{-1}) and the control groups (0.8–2.0 g•kgBM^{-1}). With inclusion of the supplement, absolute daily protein intake ranged 70–277 and 50–193 g in the protein and control groups, respectively, with a mean between-group difference of 56 g. Sixteen studies did not report daily nutrient intake.

1 *Table 3.2. Summary of studies included in the review (n = 29)*

Reference (Quality rating)	Study design Participant characteristics	Mode(s) of resistance exercise; load; sets × reps (rest period duration)	Type of protein/placebo provided [Isoenergetic (I), Non-Isoenergetic (NI)]	Protein dose and timing protocol	Daily protein intake ($g \cdot kgBM^{-1}$) excluding supplement	Daily protein intake (g) including supplement	Markers of muscle damage	Time points of measures around exercise bout	Primary findings
Baty et al. (2007) (9; excellent)	Parallel n = 34 M; young; untrained	High pull, latissimus pull-down, overhead press, bench press, leg extension, leg curl, leg press; 8RM; 3 × 8 (100 s)	Protein + CHO (1.5 + 6.2%-1.5%) Control (NI; electrolyte)	355 mL -30 min pre-Ex, 177 mL Pre & during-Ex, 355 mL Post-Ex	NR	NR	Plasma [CK and Mb]	-30 min Pre During Post +1 h +6 h +24 h	↑ [Mb] at +6 h and [CK] at +24 h in control
Bird et al. (2013) (8; good)	Crossover n = 15 M; young; trained	Back squat, deadlift, leg press, leg extension, leg curl; 8–15	Triphasic protein + CHO	8 g + 5.5 g Pre-Ex 6 g + 20 g During-Ex 22 g + 20 g	1.5	165	Serum [CK and CRP] Muscle soreness VAS (with pressure	Pre During Post +30 min +24 h	↑ [CK] AUC from pre to +30 min in control

		RM; 4 × 8–15 (90 s)	Control (NI; artificial sweetener)	Post-Ex	1.5	129	algometry) and VRS (bodyweight squats) CMJ peak power		
Buckley et al. (2010) (9; excellent)	Parallel n = 28 M; young; untrained	Eccentric unilateral knee extensions; maximal; 100	Whey protein hydrolysate (a) Whey protein isolate (b) Control (NI; flavoured water)	25 g 25 g 250 mL 0, 6 & 22 h post-Ex	NR	NR	Serum [CK] Plasma [TNF-α] Muscle soreness VAS (extended leg with 5 kg suspension) Isometric MVC knee extensor	Pre Post +1 h +2 h +6 h +24 h	↑ Isometric MVC at +6–24 h in whey protein hydrolysate compared to isolate and control
Burnley et al. (2010) (9; excellent)	Crossover n = 21 M; young; untrained	Eccentric knee extensions; 100% concentric 1RM; 10 × 10 (1 min)	Whey protein CHO (I; sugar) Control (NI; artificial sweetener	0.4 g•kgBM^{-1} 0.4 g•kgBM^{-1} 240 mL Post-Ex	0.9 1.0 1.0	107 83 78	Plasma [CK] Muscle soreness VAS (passive) Isometric MVC knee extensor and flexor	Pre Post +24 h +48 h	↔

							Knee extensor and flexor mean power		
Cockburn et al. (2008) (6; good)	Parallel n = 24 M; young; trained	Unilateral knee flexions; maximal; 6 × 10 (90 s)	Milk-based Protein + CHO (a) Semi-skimmed milk (NI) (b) CHO (NI; sports drink) Control (NI; water)	17 g 17 g 0 g 0 g All 500 mL 0 & 2 h post-Ex	NR	NR	Serum [CK and Mb] Muscle soreness VAS (passive) Isokinetic MVC knee flexion	Pre +24 h +48 h	↑ Isokinetic MVC and ↓ [CK and Mb] at +48 h in CHO-Protein and Milk compared to CHO
Cockburn et al. (2010) (4; fair)	Parallel n = 32 M; young; trained	Unilateral knee flexions; maximal; 6 × 10 (90 s)	Milk-based Protein + CHO Control (NI; water)	33 g (1000 mL) Pre-Ex (a) *or* Post-Ex (b) *or* 24 h post-Ex (c)	NR	NR	Serum [CK] Muscle soreness VAS (maximal leg flexion) Isokinetic MVC knee flexor	Pre +24 h +48 h +72 h	No significant interactions between supplement condition and time point on muscle

							Reactive strength index		damage markers
Cockburn et al. (2012) (3; poor)	Parallel n = 24 M; young; trained	Unilateral knee flexions; maximal; 6 × 10 (90 s)	Semi-skimmed milk Control (NI; water)	17 g (500 mL) [(a)] *or* 34 g (1000 mL) [(b)] Post-Ex	NR	NR	Serum [CK and Mb] Plasma [IL-6] Muscle soreness VAS (passive and during maximal leg flexion) Isokinetic MVC knee flexor	Pre +24 h +48 h +72 h	No significant interactions between supplement condition and time point on muscle damage markers

Cockburn et al. (2013) (5; fair)	Parallel n = 14 M; young; trained	Eccentric unilateral knee flexions; 6 × 10 (90 s)	Semi-skimmed milk Control (NI; water)	17 g (500 mL) Post-Ex	NR	NR	Serum [CK and Mb] Muscle soreness VAS (passive and during all performance measures) CMJ height Reactive strength index 15 m sprint Agility time Intermittent shuttle test	Pre Post +24 h +48 h +72 h	Significantly faster 15 m sprint time during shuttle test with milk than placebo
Cooke et al. (2010) (9; excellent)	Parallel n = 17 M; young; untrained	Unilateral leg press, leg extension, leg curl; 120% 1RM; 4 × 10 (3 min)	Whey protein isolate+CHO CHO (I; glucose)	1.5 g•kgBM^{-1} Post-Ex Then 27 g 4 × daily for 14 d	0.9 0.8	177 63	Plasma [CK and LDH] Isometric MVC knee extensor Maximal isokinetic knee extensor and flexor strength	Pre +30 min +1 h +2 h +4 h +24 h +48 h +72 h +96 h +7 d	↑ Isometric MVC at +72 h and +7 d with whey protein

								+14 d	
Dale et al. (2015) (9; excellent)	Parallel n = 39 M; young; untrained	Eccentric knee extension; maximal; 100	Whey protein hydrolysate (one of three forms) (a) Whey protein isolate (I) (a) Control (NI; flavoured water)	25 g Post-Ex then once daily 25 g 250 mL	NR	NR	Isometric MVC knee extensor	Pre +1 h +2 h +6 h +24 h +48 h +72 h +7 d	↔
Draganidis et al. (2017) (10; excellent)	Crossover n = 11 M; young; trained	Eccentric unilateral knee extensions; 20 × 15 (30 s)	Milk protein concentrate CHO (I; maltodextrin)	20 g 0, 3, 6 & 9 h Post-Ex Daily for 8 d 0 g	2.1 1.0	159 79	Serum [CK] Muscle soreness VAS (palpation after squats) Isometric MVC knee extensor	Pre +6 h +24 h +48 h +72 h +96 h +5 d +6 d +7 d +8 d	↑ Isometric MVC and lower muscle soreness at +1–5 d with milk protein, ↓ [CK] at +24–48 h with milk protein

Farup et al. (2014) (9; excellent)	Parallel n = 24 M; young; untrained	Eccentric unilateral knee extension; maximal; 15 × 10 (1 min)	Whey protein hydrolysate+ CHO CHO (I)	28 g + 28 g 0, 3 & 6 h Post-Ex 3× daily for 2 d 56 g	1.2 1.2	170 86	Serum [CK] Muscle soreness VAS (during single-leg squat) Isometric MVC knee extensor	Pre +24 h +48 h +72 h +96 h +7 d	↑ Muscle soreness at +96 h in whey protein
Gee et al. (2019) (10; excellent)	Parallel n = 30 M; young; trained	Back squat, bench press, deadlift, military press, bench pull; 75% 1RM; 4 × 8 (2 min)	Whey protein hydrolysate + CHO (a) Flavoured milk (I) (b) CHO (I)	33 g + 98 g 33 g + 98 g 133 g Post-Ex	NR	NR	Muscle soreness VAS Isokinetic MVC knee extensor and flexor CMJ height Medicine-ball throw distance	Pre +24 h +48 h	↔
Grubic et al. (2019) (7; good)	Crossover n = 12 M; young; trained	Bench press, incline bench press, back squat, leg press, lunges,	Whey protein + CHO food bar CHO (NI; dextrose gel)	20 g + 25 g 25 g Pre-, during, & Post-Ex	1.6 1.6	146 130	Serum [CK, IL-8 and TNF-α] Muscle soreness VAS	Pre During Post +48 h	↔

		leg extension, latissimus pulldown, row, bicep curls, triceps pulldown, preacher curls; 70% 1RM; 3 × 10 (2 min) Followed by sprint intervals					(with pressure algometry) Isokinetic MVC knee extensor and flexor		
Hasegawa et al. (2014) (4; fair)	Crossover n = 6 M; young; untrained	Leg extension, leg press, row, chest fly; 80% 1RM; 3 × 10 (1 min)	Egg white protein (a) Soy protein (I) (b) Control (NI; water)	20 g 20 g Pre-Ex	0.9 0.8 0.8	76 70 50	Serum [CK] Muscle soreness VAS (passive)	Pre Post +30 min +24 h +48 h +72 h	↔
Hirose et al. (2013) (6; good)	Crossover	Calf-raise; 100% BM; 5 × 15 (60 s)	Milk peptide Control (NI; no supplement)	5 g Pre- & Post-Ex	NR	NR	Plasma [CK] Muscle soreness VAS	Pre Post +12 h	↓ [CK] at +72 h and +5

	n = 6 M; young; untrained			Twice daily for 5 d			(palpation and unloaded extension)	+24 h +48 h +72 h +96 h +5 d +8 d	d with milk peptide, ↓ Muscle soreness with milk peptide
Hoffman et al. (2010) (8; good)	Parallel n = 15 M; young; trained	Back squat, deadlift, barbell lunge; 80% 1RM; 4 × 10 (90 s)	Protein blend (collagen, whey, and casein isolates, plus 250 mg BCAAs) CHO (NI; maltodextrin)	42 g Pre- & Post-Ex for 3 d	2.0 2.0	277 193	Serum [CK] Muscle soreness VRS Squat peak power	Pre Post +24 h +48 h	↔
Ives et al. (2017) (7; good)	Parallel n = 60 M; young; untrained	Eccentric unilateral knee extension; maximal; 100	Whey protein hydrolysate CHO (I; sugar flavoured water)	31 g 0, 6 & 22 h Post-Ex 250 mL	1.3 1.4	189 96	Muscle soreness VAS (extended leg with and without 5 kg suspension) Isometric MVC knee extensor	Pre Post +1 h +2 h +6 h +24 h	↑ Peak isokinetic MVC at +24 h with protein compared to CHO

							Isokinetic MVC knee extensor Thigh circumference		
Karakus & Akkurt (2020) (5; fair)	Parallel n = 24 M; young; trained	Bench press, chest fly, reverse fly, shoulder press, triceps pushdown, bicep curl, back squat, leg press, leg extension, leg flexion, adductor, calf press; 80%, 90% and 100% 1RM; 3 × 10	Whey protein Control (NI; no supplement)	35 g Post-Ex then daily	1.0 1.0	105 70	Serum [CK and Mb] Muscle soreness VAS	Pre Post +24 h +48 h +72 h	↑ Muscle soreness at +24–72 h with protein

Kim et al. (2017) (7; good)	Parallel n = 32 M; young; untrained	Eccentric elbow flexions; 2 × 25	Whey protein Control (NI; no supplement)	1.5 g•kgBM^{-1} Pre-Ex (a) *or* Post-Ex (b) *or* Pre- & Post-Ex (b)	NR	NR	Serum [CK] Muscle soreness VAS Isometric MVC elbow flexor ROM elbow flexor	Pre Post +24 h +48 h +72 h +96 h	↔
Naclerio et al. (2020) (9; excellent)	Crossover n = 10 M; young; trained	Bench press, back squat, row, lunge, deadlift, box step-up, hang clean; 40–60% 1RM; 3 × 15 (30 s) 3 consecutive days	Protein blend (whey isolate, beef hydrolysate, glutamine + CHO) CHO (I; maltodextrin)	18 g + 38 g Post-Ex for 3 d 55 g	1.7 1.7	145 127	Muscle soreness VAS (bodyweight squat) Bench press 1RM and maximal power CMJ height	Pre +1 h +24 h +48 h	↑ CMJ height at +1 h, maximal strength at +24 h, and maximal power at +24–48 h with protein
Nieman et al. (2020) (10; excellent)	Parallel	Bench press, eccentric latissimus	Whey protein isolate (a)	0.3 g•kgBM^{-1}	NR	NR	Serum [CK, Mb, LDH and CRP]	Pre Post +24 h	↓ [CK] at +72–96 h and [Mb] at +48–

	n = 92 M; young; untrained	pulldown, eccentric leg extension, eccentric leg curl, split squats, and various unloaded exercises; ≤maximal; 2–4 × 3–15 (30–60 s)	Pea protein isolate (I) (b) Control (NI; water)	0.3 g•kgBM^{-1} 237 mL Pre- & Post-Ex and pre-sleep for 4 d			Muscle soreness VAS Isometric MVC deadlift dynamometer Bench press repetitions CMJ height 30-s Wingate	+48 h +72 h +96 h	96 h with whey protein compared to water, ↔ between pea protein and water
Philpott et al. (2018) (7; good)	Parallel n = 36 M; young; trained	Eccentric unilateral knee flexion; 12 sets (each leg) (1 min)	Whey protein + Leucine + CHO CHO (NI)	15 + 1.8 + 20 g Twice daily for 6 wk pre-Ex 24 g	NR	NR	Serum [CK] Plasma [CRP] Muscle soreness VAS (passive and unloaded leg extension) Isokinetic MVC knee flexor	Pre +24 h +48 h +72 h	↔

							Yo-Yo intermittent endurance test Soccer passing skills test		
Rankin et al. (2015) (7; good)	Parallel n = 32 (16M, 16F); young; trained	Eccentric knee flexion; maximal; 6 × 10 (90 s)	Milk CHO (I)	17 g (500 mL) Post-Ex 500 mL	NR	NR	Serum [CK] Muscle soreness VAS (passive and maximal leg flexion) Isokinetic MVC knee flexor CMJ height 20 m sprint	Pre +24 h +48 h +72 h	No significant interactions between supplement condition and time point on muscle damage markers
Samadi et al., (2012) (6; good)	Parallel n = 28 M; young; untrained	Leg press, leg curl, leg extension, shoulder press, latissimus pulldown,	9% whey protein + CHO in various ratios: 1:4 1:3 1:2	24 g divided into 4 doses 2.5 mL•kgBM^{-1} solution Pre-Ex and 3 doses	NR	NR	Plasma [CK and Mb] Muscle soreness VRS (passive)	Pre +1 h +24 h +48 h	↑ [CK and Mb] at +24 h in control

		bench press, bicep curl; 70–75% 1RM; 3 × 8 (1 min)	Control (NI; artificial sweetener)	during Ex at 15-min intervals					
Saracino et al. (2020) (9; excellent)	Parallel n = 32 M; middle-aged; untrained	Eccentric knee extensions and flexions; maximal; 5 × 15 (2 min)	Whey protein hydrolysate (a) Whey protein isolate (b) Rice and pea protein (c) Control (NI)	40 g Pre-sleep before Ex and for 2 d post-Ex and 25 g bolus post-Ex	1.1 1.1 1.1 1.1	155 156 151 84	Plasma [CK, IL-6 and IL-10] Muscle soreness VAS (unloaded leg extension) Isometric MVC knee extensor and flexor Isokinetic MVC knee extensor and flexor Thigh circumference	Pre +24 h +48 h +72 h	↔

West et al. (2017) (9; excellent)	Crossover n = 12 M; young; trained	Bench press, latissimus pulldown, barbell shoulder press, row, leg press, leg extension; 75% 1RM; 4 × 10 (2 min) Or non-exercising control	Whey protein CHO (I) Control (No supplement)	25 g 0 & 10 h Post-Ex	NR	NR	Isometric MVC knee extensor CMJ performance Knee extensor repetitions to failure at 75% 1RM 30 s Wingate	Pre Post +10 h +24 h	Small-to-moderate beneficial effects on isometric MVC, anaerobic power, and CMJ at +10 h and moderate beneficial effects on isometric MVC, anaerobic power and muscular endurance at +24 h with protein
White et al. (2008)	Parallel	Eccentric unilateral	Whey protein + CHO	23 + 75 g Pre-Ex [(a)] *or*	NR	NR	Serum [CK]	Pre +6 h	↔

(9; excellent)	n = 27 M; young; untrained	knee extensions; maximal; 5 × 10 (1 min)	Control (NI; artificial sweetener)	Post-Ex (b) Pre- & Post-Ex			Muscle soreness VAS Isometric MVC knee extensor	+24 h +48 h +72 h +96 h	
Wojcik et al. (2001) (6; good)	Parallel n = 26 M; young; untrained	Eccentric knee extension; 120% of concentric 1RM; 10 × 10 (1 min)	Flavoured milk protein + CHO CHO (I; Gatorade) Control (NI; artificial sweetener)	25 + 70 g 0 and 2 h post-Ex 95 g 0 g	1.5 1.5 1.5	164 113 113	Serum [CK, IL-1, IL-6 and TNF-α] Muscle soreness VRS (passive) Isometric MVC knee extensor	Pre Post +3 h +6 h +24 h +48 h +72 h +96 h +5 d +6 d	↔

Arrows indicate a significant difference between groups (↑ = higher, ↓ = lower, ↔ = no significant differences); methodological quality rating categorised as 0–3 = 'poor', 4–5 = 'fair', 6–8 'good', and 9–10 = 'excellent'; participant age is classified as young (18–39 y), middle-aged (40–64 y), and older adults (≥ 65 y) (Schoenfeld et al., 2017$_a$); subscripts (a), (b), and (c) denote separate trials within a study; M = males; F = females; 1RM = one repetition maximum; CHO = carbohydrate; BCAAs = branched chain amino acids; Ex = exercise; NR = not reported; CK = creatine kinase; Mb = myoglobin; LDH = lactate dehydrogenase; IL = interleukin; TNF-α = tumor necrosis factor-alpha; CRP = C-reactive protein; VAS = visual analogue scale; VRS = visual rating scale; CMJ = counter movement jump; MVC = maximal voluntary contraction; ROM = range of motion; PPT = pressure pain threshold.

Table 3.3. Mean percent change values from trials not included in the meta-analyses

		Mean percent change (range)				
Variable	Group	Pre-Post	Pre-24 h	Pre-48 h	Pre-72 h	Pre-96 h
Isometric MVC	Protein	-29.9 – -2.4	-31.9 – 0.0	-32.3 – -0.7	-27.3 – 2.4	-23.0 – 6.1
	Control	-36.7 – -2.6	-38.0 – -3.2	-32.1 – -3.8	-32.6 – -2.6	-22.2 – 3.8
Isokinetic MVC	Protein	-24.2 – -1.5	-26.2 – 0.6	-42.1 – -3.2	–	–
	Control	-20.0 – 0.7	-25.5 – -8.8	-33.7 – -2.1	–	–
CK	Protein	2.0 – 166	29.8 – 1660.2	15.9 – 7532.3	152.0 – 18769.1	166.6 – 33222.0
	Control	-7.9 – 283.7	37.1 – 1605.7	42.7 – 3770.8	363.8 – 10471.4	325.3 – 13907.6

Time is in relation to the exercise bout; MVC = maximal voluntary contraction; CK = creatine kinase

3.4.2. Isometric Maximal Voluntary Contraction

Baseline isometric MVC ranged 133.7–292.4 and 125.3–314.0 Nm in protein and control groups, respectively. Three trials did not report baseline data (Farup et al., 2014; Kim et al., 2017; West et al., 2017). Meta-analyses for isometric MVC change from baseline between protein and control groups were conducted at <24 h (k = 8), 24 h (k = 11), 48 h (k = 8), 72 h (k = 8), and 96 h (k = 6). Overall ESs were small and non-significant at <24–72 h (*Figures 3.2.a–d)*. An influential trial was detected at 48 h (Dale et al., 2015)$_b$ and its removal resulted in significant medium positive effects of protein (pooled ES = 0.564 [0.049, 1.080]). There was an overall beneficial effect of protein at 96 h (ES = 0.563 [0.232, 0.894]) (*Figure 3.2.e*).

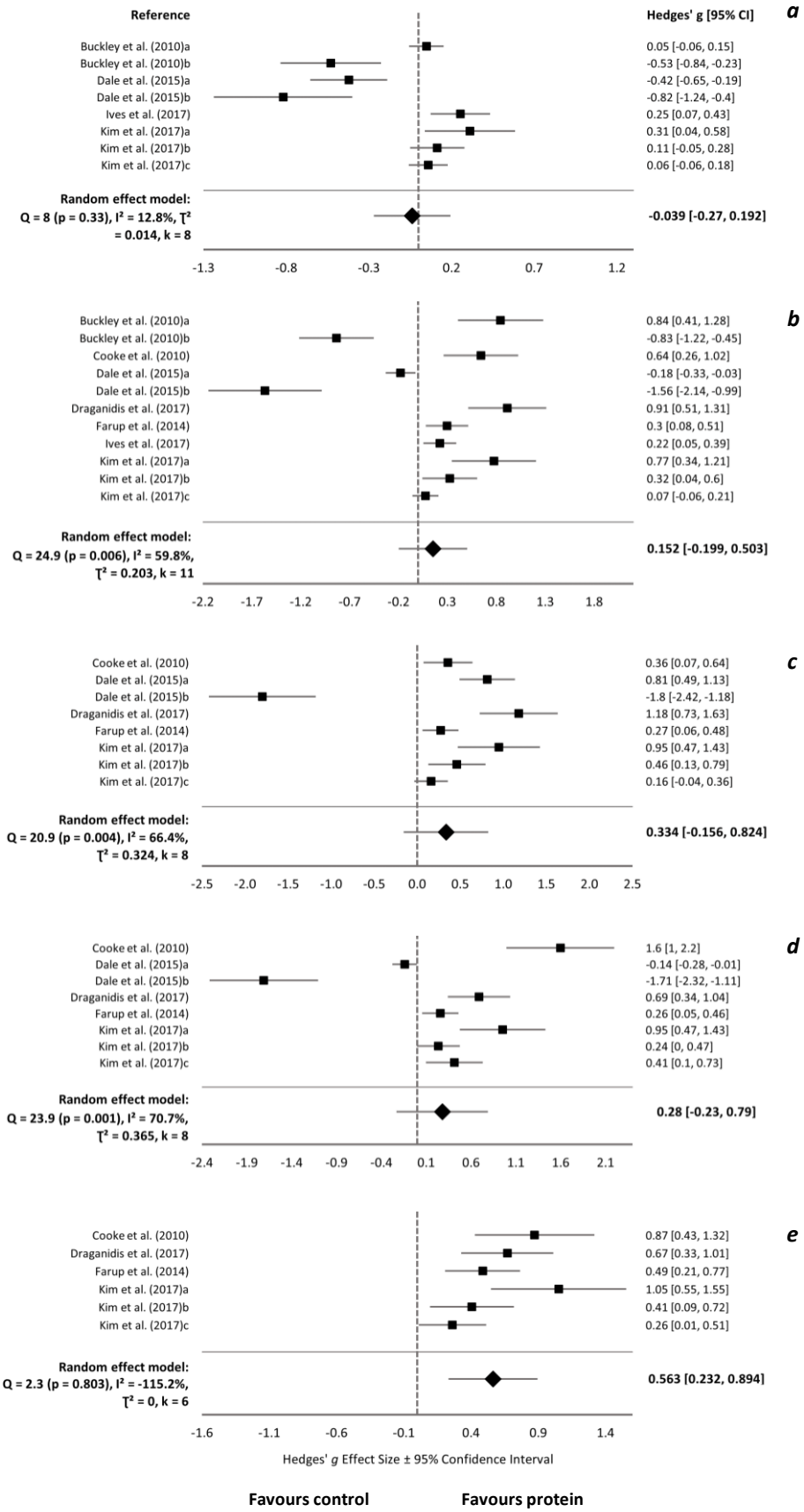

a
Reference
Hedges' g [95% CI]
Buckley et al. (2010)a
Buckley et al. (2010)b
Dale et al. (2015)a
Dale et al. (2015)b
Ives et al. (2017)
Kim et al. (2017)a
Kim et al. (2017)b
Kim et al. (2017)c
0.05 [-0.06, 0.15]
-0.53 [-0.84, -0.23]
-0.42 [-0.65, -0.19]
-0.82 [-1.24, -0.4]
0.25 [0.07, 0.43]
0.31 [0.04, 0.58]
0.11 [-0.05, 0.28]
0.06 [-0.06, 0.18]
Random effect model:
Q = 8 (p = 0.33), I² = 12.8%, T² = 0.014, k = 8
-0.039 [-0.27, 0.192]
-1.3 -0.8 -0.3 0.2 0.7 1.2
b
Buckley et al. (2010)a
Buckley et al. (2010)b
Cooke et al. (2010)
Dale et al. (2015)a
Dale et al. (2015)b
Draganidis et al. (2017)
Farup et al. (2014)
Ives et al. (2017)
Kim et al. (2017)a
Kim et al. (2017)b
Kim et al. (2017)c
0.84 [0.41, 1.28]
-0.83 [-1.22, -0.45]
0.64 [0.26, 1.02]
-0.18 [-0.33, -0.03]
-1.56 [-2.14, -0.99]
0.91 [0.51, 1.31]
0.3 [0.08, 0.51]
0.22 [0.05, 0.39]
0.77 [0.34, 1.21]
0.32 [0.04, 0.6]
0.07 [-0.06, 0.21]
Random effect model:
Q = 24.9 (p = 0.006), I² = 59.8%, T² = 0.203, k = 11
0.152 [-0.199, 0.503]
-2.2 -1.7 -1.2 -0.7 -0.2 0.3 0.8 1.3 1.8
c
Cooke et al. (2010)
Dale et al. (2015)a
Dale et al. (2015)b
Draganidis et al. (2017)
Farup et al. (2014)
Kim et al. (2017)a
Kim et al. (2017)b
Kim et al. (2017)c
0.36 [0.07, 0.64]
0.81 [0.49, 1.13]
-1.8 [-2.42, -1.18]
1.18 [0.73, 1.63]
0.27 [0.06, 0.48]
0.95 [0.47, 1.43]
0.46 [0.13, 0.79]
0.16 [-0.04, 0.36]
Random effect model:
Q = 20.9 (p = 0.004), I² = 66.4%, T² = 0.324, k = 8
0.334 [-0.156, 0.824]
-2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0 2.5
d
Cooke et al. (2010)
Dale et al. (2015)a
Dale et al. (2015)b
Draganidis et al. (2017)
Farup et al. (2014)
Kim et al. (2017)a
Kim et al. (2017)b
Kim et al. (2017)c
1.6 [1, 2.2]
-0.14 [-0.28, -0.01]
-1.71 [-2.32, -1.11]
0.69 [0.34, 1.04]
0.26 [0.05, 0.46]
0.95 [0.47, 1.43]
0.24 [0, 0.47]
0.41 [0.1, 0.73]
Random effect model:
Q = 23.9 (p = 0.001), I² = 70.7%, T² = 0.365, k = 8
0.28 [-0.23, 0.79]
-2.4 -1.9 -1.4 -0.9 -0.4 0.1 0.6 1.1 1.6 2.1
e
Cooke et al. (2010)
Draganidis et al. (2017)
Farup et al. (2014)
Kim et al. (2017)a
Kim et al. (2017)b
Kim et al. (2017)c
0.87 [0.43, 1.32]
0.67 [0.33, 1.01]
0.49 [0.21, 0.77]
1.05 [0.55, 1.55]
0.41 [0.09, 0.72]
0.26 [0.01, 0.51]
Random effect model:
Q = 2.3 (p = 0.803), I² = -115.2%, T² = 0, k = 6
0.563 [0.232, 0.894]
-1.6 -1.1 -0.6 -0.1 0.4 0.9 1.4
Hedges' g Effect Size ± 95% Confidence Interval
Favours control
Favours protein

Figure 3.2. Forest plot of Hedges' g effect sizes with 95% confidence intervals for the effect of protein supplementation compared to control on the change from baseline in isometric maximal voluntary contraction at a. <24 h post-exercise, b. 24 h post-exercise, c. 48 h post-exercise, d. 72 h post-exercise, and e. 96 h post-exercise. A positive effect size indicates a beneficial effect of protein supplementation compared to control. All eligible trials, including outliers, are presented and included in the analysis.

3.4.3. Isokinetic Maximal Voluntary Contraction

Isokinetic MVC at baseline ranged 74.5–188.0 and 72.1–183.0 Nm in protein and control groups, respectively, and was not reported in 2 studies (Cockburn et al., 2010; Philpott et al., 2018). Meta-analyses were conducted at 24 h (k = 3), 48 h (k = 8), and 72 h (k = 8). Overall ESs were small-medium in favour of protein and reached statistical significance at all time-points (*Figures 3.3.a–c*). Philpott et al. (2018) was identified as influential and its removal resulted in non-significant overall ESs at 48 (0.319 [-0.036, 0.675] and 72 h (0.371 [-0.08, 0.822]). There was no clear impact of protein type, time or duration of supplementation, muscle group exercised, contraction type, or the training status of participants on the change in isometric and isokinetic MVC at 24 h (*supplementary Figure S3.1.a.*).

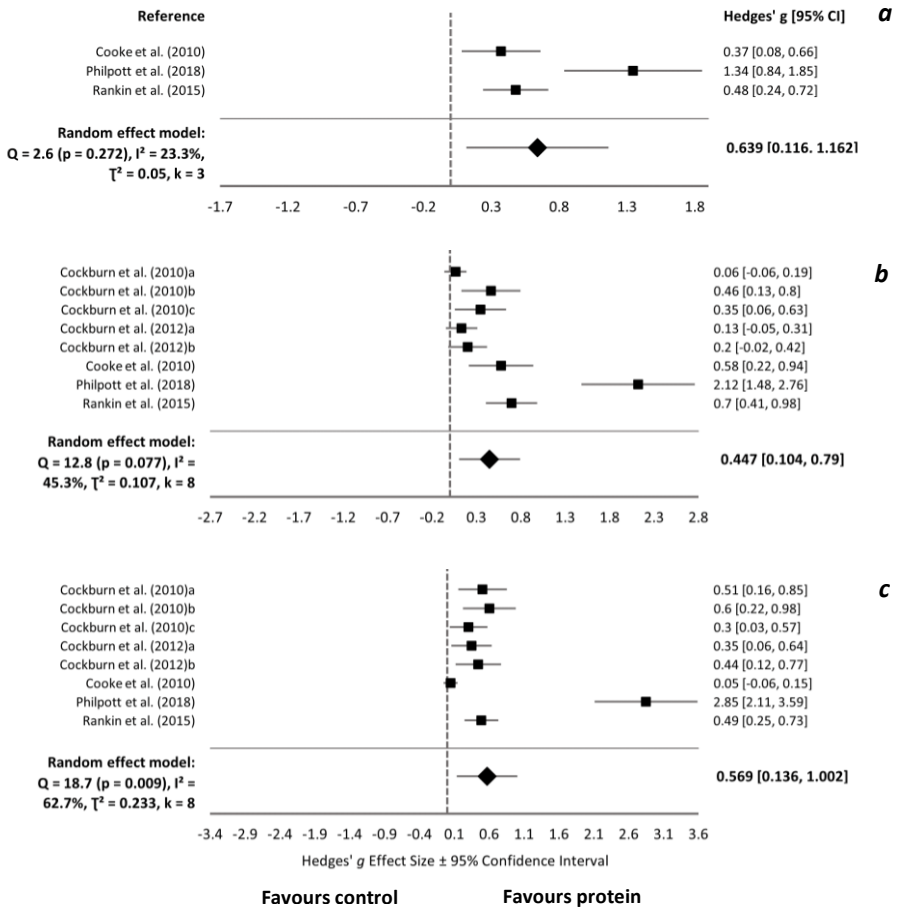


Figure 3.3. Forest plot of Hedges' g effect sizes with 95% confidence intervals for the effect of protein supplementation compared to control on the change from baseline in isokinetic maximal voluntary contraction at a. 24 h post-exercise, b. 48 h post-exercise, and c. 72 h post-exercise. A positive effect size indicates a beneficial effect of protein supplementation compared to control. All eligible trials, including outliers, are presented and included in the analysis.

3.4.4. Creatine Kinase

Baseline [CK] ranged 33.7–307.0 and 43.5–540.5 IU•L^{-1} in protein and control groups, respectively. There were insufficient data to meta-analyse trials at <24, 24, and 96 h. At 48 h, 6 trials produced significant medium-large positive effects of protein and the overall effect was borderline significant (ES = 0.836 [-0.001, 1.673]) (*Figure 3.4.a*). One influential trial (Cockburn et al., 2013) produced large negative effects (-1.88 [-2.59, -1.16]) and, once removed, the overall effect became large in favour of protein (1.252 [0.354, 2.151]). There were large positive effects of protein at 72 h (overall ES = 1.335 [0.294, 2.376]) (*Figure 3.4.b*). Removal of one influential trial (Rankin et al., 2015) resulted in non-significant overall effects (0.952 [-0.170, 2.075]).

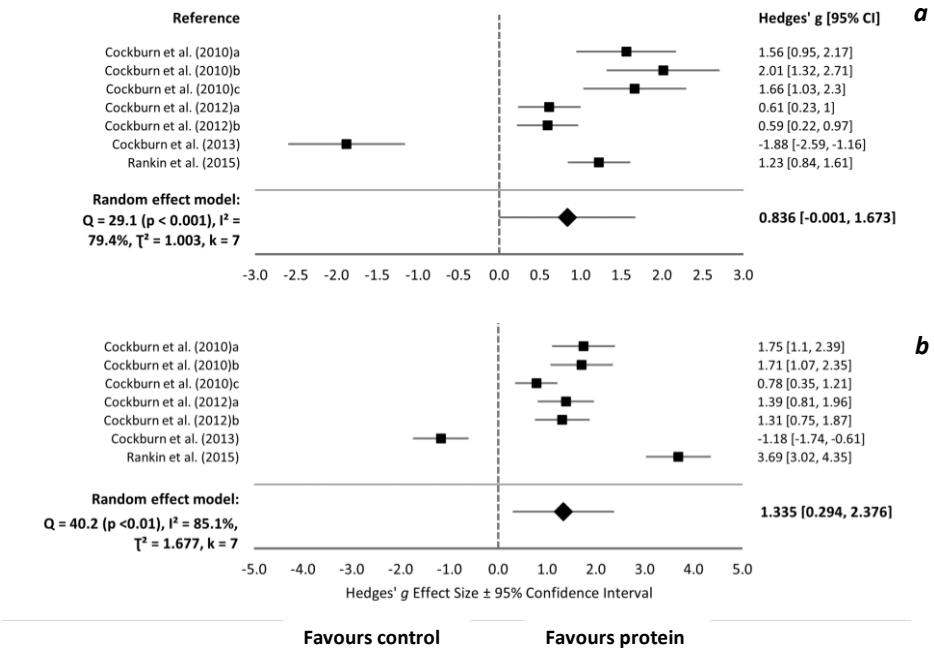
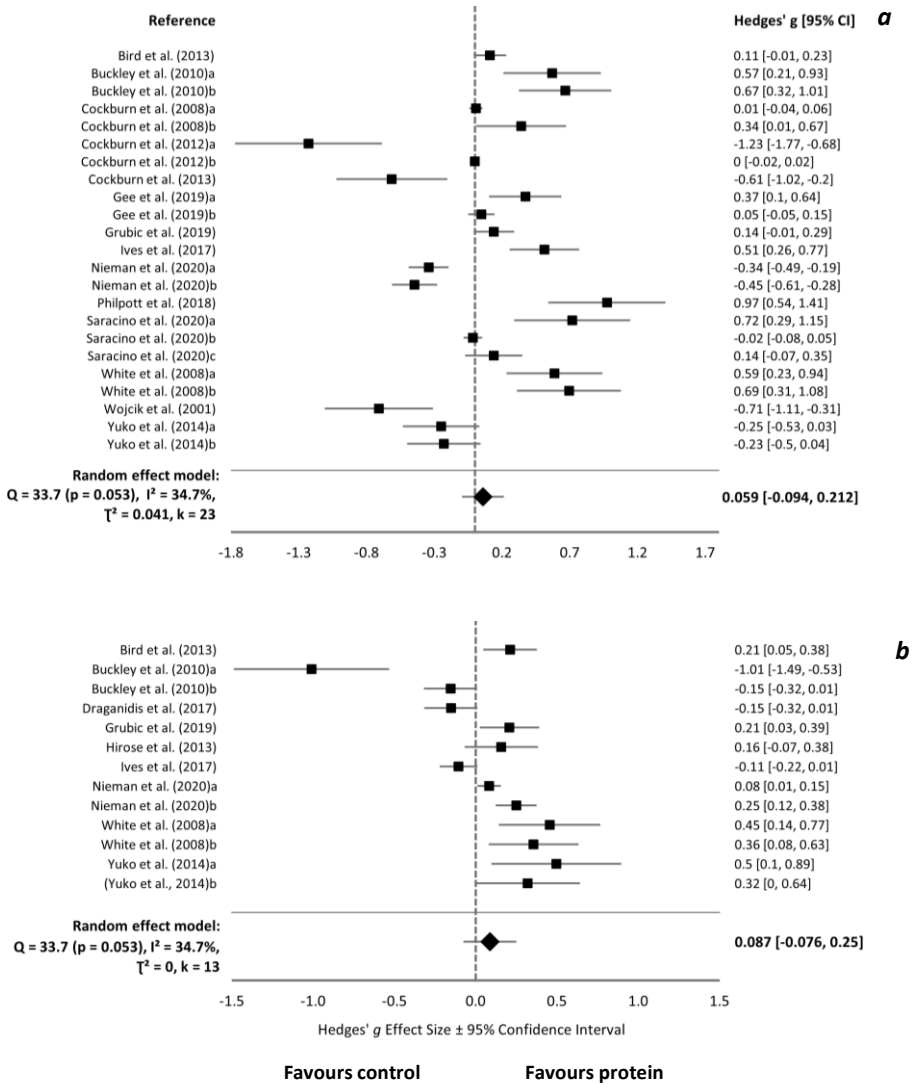
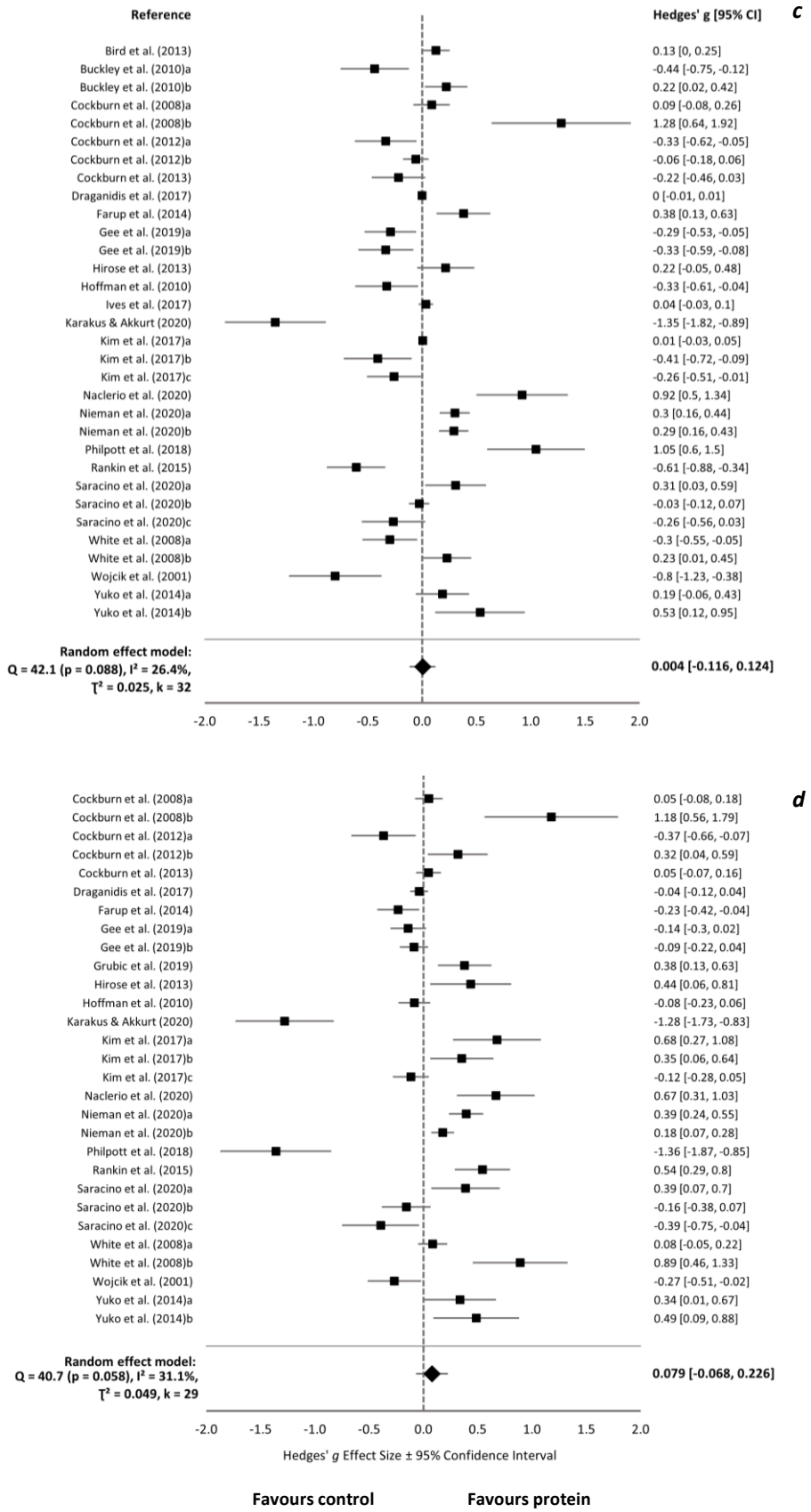


Figure 3.4. Forest plot of Hedges' g effect sizes with 95% confidence intervals for the effect of protein supplementation compared to control on the change from baseline in serum creatine kinase concentration at a. 48 h post-exercise and b. 72 h post-exercise. A positive effect size indicates a beneficial effect of protein supplementation compared to control. All eligible trials, including outliers, are presented and included in the analysis.

3.4.5. Muscle Soreness

Baseline muscle soreness ranged 0.0–27.9 and -0.5–31.4 mm in protein and control groups, respectively, and was not reported in one study (Cockburn et al., 2010). Meta-analyses were conducted at baseline (k = 23), <24 h (k = 13), 24 h (k = 32), 48 h (k = 29), 72 h (k = 22), and 96 h (k = 11). There was no overall effect of supplement group on muscle soreness at any time-point (*Figures 3.5.a–f*). At 72 h, one influential trial was identified (Philpott et al., 2018) and, upon removal, a significant positive effect of protein was found (overall ES = 0.230 [0.054, 0.406]). Protein supplementation appears more beneficial for muscle soreness in untrained individuals, following concentric exercise, and with a single day of supplementation (*supplementary Figure S3.1.b*).




c
Reference
Hedges' g [95% CI]
Bird et al. (2013) 0.13 [0, 0.25]
Buckley et al. (2010)a -0.44 [-0.75, -0.12]
Buckley et al. (2010)b 0.22 [0.02, 0.42]
Cockburn et al. (2008)a 0.09 [-0.08, 0.26]
Cockburn et al. (2008)b 1.28 [0.64, 1.92]
Cockburn et al. (2012)a -0.33 [-0.62, -0.05]
Cockburn et al. (2012)b -0.06 [-0.18, 0.06]
Cockburn et al. (2013) -0.22 [-0.46, 0.03]
Draganidis et al. (2017) 0 [-0.01, 0.01]
Farup et al. (2014) 0.38 [0.13, 0.63]
Gee et al. (2019)a -0.29 [-0.53, -0.05]
Gee et al. (2019)b -0.33 [-0.59, -0.08]
Hirose et al. (2013) 0.22 [-0.05, 0.48]
Hoffman et al. (2010) -0.33 [-0.61, -0.04]
Ives et al. (2017) 0.04 [-0.03, 0.1]
Karakus & Akkurt (2020) -1.35 [-1.82, -0.89]
Kim et al. (2017)a 0.01 [-0.03, 0.05]
Kim et al. (2017)b -0.41 [-0.72, -0.09]
Kim et al. (2017)c -0.26 [-0.51, -0.01]
Naclerio et al. (2020) 0.92 [0.5, 1.34]
Nieman et al. (2020)a 0.3 [0.16, 0.44]
Nieman et al. (2020)b 0.29 [0.16, 0.43]
Philpott et al. (2018) 1.05 [0.6, 1.5]
Rankin et al. (2015) -0.61 [-0.88, -0.34]
Saracino et al. (2020)a 0.31 [0.03, 0.59]
Saracino et al. (2020)b -0.03 [-0.12, 0.07]
Saracino et al. (2020)c -0.26 [-0.56, 0.03]
White et al. (2008)a -0.3 [-0.55, -0.05]
White et al. (2008)b 0.23 [0.01, 0.45]
Wojcik et al. (2001) -0.8 [-1.23, -0.38]
Yuko et al. (2014)a 0.19 [-0.06, 0.43]
Yuko et al. (2014)b 0.53 [0.12, 0.95]
Random effect model:
Q = 42.1 (p = 0.088), I² = 26.4%,
τ² = 0.025, k = 32
0.004 [-0.116, 0.124]
-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0
d
Cockburn et al. (2008)a 0.05 [-0.08, 0.18]
Cockburn et al. (2008)b 1.18 [0.56, 1.79]
Cockburn et al. (2012)a -0.37 [-0.66, -0.07]
Cockburn et al. (2012)b 0.32 [0.04, 0.59]
Cockburn et al. (2013) 0.05 [-0.07, 0.16]
Draganidis et al. (2017) -0.04 [-0.12, 0.04]
Farup et al. (2014) -0.23 [-0.42, -0.04]
Gee et al. (2019)a -0.14 [-0.3, 0.02]
Gee et al. (2019)b -0.09 [-0.22, 0.04]
Grubic et al. (2019) 0.38 [0.13, 0.63]
Hirose et al. (2013) 0.44 [0.06, 0.81]
Hoffman et al. (2010) -0.08 [-0.23, 0.06]
Karakus & Akkurt (2020) -1.28 [-1.73, -0.83]
Kim et al. (2017)a 0.68 [0.27, 1.08]
Kim et al. (2017)b 0.35 [0.06, 0.64]
Kim et al. (2017)c -0.12 [-0.28, 0.05]
Naclerio et al. (2020) 0.67 [0.31, 1.03]
Nieman et al. (2020)a 0.39 [0.24, 0.55]
Nieman et al. (2020)b 0.18 [0.07, 0.28]
Philpott et al. (2018) -1.36 [-1.87, -0.85]
Rankin et al. (2015) 0.54 [0.29, 0.8]
Saracino et al. (2020)a 0.39 [0.07, 0.7]
Saracino et al. (2020)b -0.16 [-0.38, 0.07]
Saracino et al. (2020)c -0.39 [-0.75, -0.04]
White et al. (2008)a 0.08 [-0.05, 0.22]
White et al. (2008)b 0.89 [0.46, 1.33]
Wojcik et al. (2001) -0.27 [-0.51, -0.02]
Yuko et al. (2014)a 0.34 [0.01, 0.67]
Yuko et al. (2014)b 0.49 [0.09, 0.88]
Random effect model:
Q = 40.7 (p = 0.058), I² = 31.1%,
τ² = 0.049, k = 29
0.079 [-0.068, 0.226]
-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0
Hedges' g Effect Size ± 95% Confidence Interval
Favours control
Favours protein

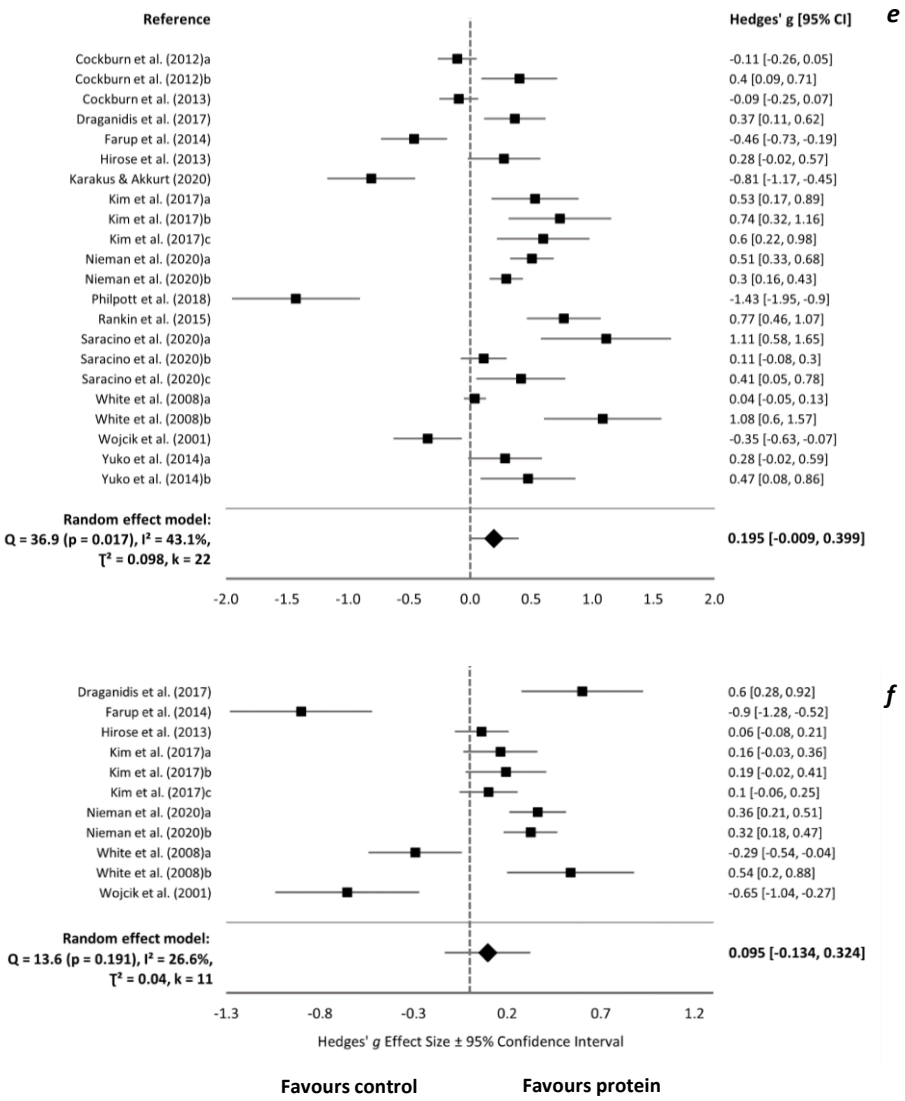


Figure 3.5. Forest plot of Hedges' g effect sizes with 95% confidence intervals for the effect of protein supplementation compared to control on muscle soreness ratings at a. pre-exercise, b. <24 h post-exercise, c. 24 h post-exercise, d. 48 h post-exercise, e. 72 h post-exercise, and f. 96 h post-exercise. A positive effect size indicates a beneficial effect of protein supplementation compared to control. All eligible trials, including outliers, are presented and included in the analysis.

3.5.0. Discussion

Peri-exercise protein consumption has beneficial effects for preserving acute muscle strength and blunting [CK] following muscle damaging resistance exercise in young males. Reductions in isokinetic MVC were significantly attenuated by protein in all 8 trials at ≥1 time-point, with no negative effects of protein consumption. Nine out of 11 trials were in favour of consuming protein for reducing isometric strength loss compared to control products at ≥1 time-point. Likewise, only one trial failed to demonstrate a positive effect of protein for attenuating post-

exercise [CK] elevations. Protein consumption is unlikely beneficial for reducing post-exercise muscle soreness in young males, as zero-small effects were observed (ES range = 0.004–0.195). This review could not establish the impact of protein supplementation on EIMD in females due to a lack of studies conducted with females or both sexes.

Despite its frequent assessment, the efficacy of protein consumption for muscle soreness management is confounding. Less than half of trials reviewed reported a benefit of protein for reducing post-exercise muscle soreness at 48 h. These conflicting data reflect the existing limited understanding of the mechanisms of exercise-induced muscle soreness, alongside its subjectivity and susceptibility to other physiological and psychological influencers (e.g. mood, sleep quality, hormonal status) (Melzack, 1982). However, this review identified that males untrained in resistance exercise are more likely to respond positively to protein supplementation than trained males. Similarly, protein supplementation more frequently reduced symptoms of muscle soreness following concentric than eccentric exercise. Therefore, both training status and muscle contraction type may influence muscle soreness responses to protein supplementation. Investigating these factors may allude to muscle soreness mechanisms, although current understanding is hindered by the varied assessment methods used, for example, different rating scales, participant positioning, pressure algometry, muscle palpation, passively, or actively with mixed forms of activity. Such inconsistencies might explain why muscle soreness is argued to poorly reflect EIMD (Nosaka et al., 2002_a). Until optimal and consistent methods for assessing muscle soreness are employed, data should be treated cautiously.

Post-exercise muscle strength decrements debilitate future exercise quality for up to 7 days (Byrne & Eston, 2002; Farup et al., 2014). This review has demonstrated that peri-exercise protein consumption can reduce muscle strength loss and accelerate the recovery of muscle function. Therefore, high-quality exercise may be resumed faster with the aid of protein consumption compared to carbohydrate-based or no peri-exercise nutritional strategy. Currently, the use of protein as an exercise nutrition strategy is recommended for muscle recovery, repair, and growth, due to its stimulatory effects on post-exercise MPS rates (Tipton et al., 1999), which are augmented by protein consumption relative to exercise alone (Moore et al., 2009). It follows that peri-exercise protein consumption may be recommended as a multi-purpose nutritional aid – assisting in the management of muscle damage and repair processes. This dual-target strategy could help lift financial, time, and resource constraints, as opposed to following multiple dietary and/or physiotherapeutic strategies serving individual purposes.

It is unclear how protein supplementation may reduce muscle strength decrements following exercise and here, protein ingestion was only beneficial for isometric MVC at 96 h post-exercise. One explanation is that MPS is augmented by protein relative to carbohydrate ingestion during the later (72–168 h) but not earlier (24–72 h) recovery period after EIMD (Pavis et al., 2021). By this means, repair and remodelling of muscle proteins and restoration of muscle function may occur at an accelerated rate with protein supplementation. However, it is difficult to explain why protein supplementation benefited isokinetic but not isometric MVC at 24–72 h post-exercise. These outcomes appear predominantly due to one influential study (Philpott et al., 2018), though nonetheless could relate to the magnitude of strength decline, which was typically lower for isokinetic compared with isometric MVC. The pathways by which protein ingestion acts to attenuate EIMD warrant investigation, though notably, factors other than post-exercise amino acid availability play a role (Pavis et al., 2021).

Much research on protein nutrition, particularly related to MPS, has sought to establish the optimal type, dose, and timing of protein consumption to maximally stimulate post-exercise MPS rates (Witard et al., 2016). Although, this has not been the case for EIMD. The present review identified no discernible effects of protein ingestion timing, type, dosage (ranged 5–104 g), or days of supplementation on EIMD (*Figure S3.1*). However, other inter-study differences in methodological design, for example the exercise protocol, sample demographics, and measurement tools may limit the ability to compare protein supplementation protocols between trials.

This review identified few trials that compared protein supplementation strategies while being matched for other methodological detail. Before or after eccentric leg extension exercise, 23 g of whey protein plus 75 g of carbohydrate was provided to untrained males by White et al. (2008), which had no impact on EIMD, irrespective of supplementation timing. Likewise, there were no differences in EIMD between groups when a large dose (1.5 g•kgBM^{-1}) of whey protein was consumed pre, post, or pre and post exercise by untrained males (Kim et al., 2017). In a series of experiments with comparable methodological design, the impact of various milk protein feeding strategies on EIMD in trained males performing leg-based resistance exercise was examined by Cockburn and colleagues. No significant interactions between EIMD markers and milk protein timing (pre, post, or 24 h post-exercise), dosage (17 or 34 g), or type (milk or flavoured milk plus carbohydrate) were found by these authors (Cockburn et al., 2008; 2010; 2012). Similarly, the ingestion of flavoured milk relative to an isonitrogenous dose of whey protein hydrolysate by trained males did not impact muscle damage following whole-body

resistance exercise (Gee et al., 2019). Conversely, the type of ingested protein was indicated to influence EIMD by Buckley et al. (2010).

It was noted by Buckley and colleagues (2010) that maximal isometric strength was preserved at 6–24 h following maximal eccentric exercise when hydrolysed whey protein, but not isolated whey protein or flavoured water, was consumed post-exercise by untrained males. This finding was unusual, especially as there were no between-group differences in peak strength decrements at 0–2 h post-exercise, nor in other EIMD markers. Another anomaly was the tendency for maximal strength to undergo a second decrease from 6 to 24 h following whey isolate ingestion, while maximal strength of the placebo group gradually returned to baseline. It was proposed by the authors that the hydrolysed whey protein accelerated strength recovery relative to non-hydrolysed protein by means of stimulating muscle repair processes. However, this theory seems unlikely, given that isolated whey protein stimulates increased post-exercise MPS rates (Borack et al., 2016; Burd et al., 2012; Macnaughton et al., 2016; Yang et al., 2012). Furthermore, comparable isometric strength reductions and recovery rates after eccentric exercise with ingested whey hydrolysate, whey isolate, and flavoured water were observed in a similar study (Dale et al., 2015). Supporting data (Saracino et al., 2020) make it challenging to explain the outcomes of Buckley (2010); thus, the impact of protein hydrolysis on EIMD warrants further investigation. To ascertain the importance of protein feeding type, timing, and dosage for the management of EIMD, further studies with comparable methodologies are required. Due to the apparent lack of difference between isolated and whole-food sources of protein, future studies should adopt a food-first approach where feasible.

The food-first approach aids the achievement of multiple nutrient requirements; however, meeting protein intake goals using this approach may be challenging for some protein types. Accordingly, the ‘food first but not always food only’ approach has been advocated (Close et al., 2022). Plant-based proteins present a challenge, as they necessitate consumption of larger food volumes to achieve protein requirements. For example, 20 g of milk protein can be obtained through ~200 g of dairy yoghurt, while ~500 g of soya yoghurt is required to obtain 20 g of soy protein. Alternatively, a single-serve of isolated soy protein conveniently provides an isonitrogenous dose. Plant-based diets are growing in popularity, due to various health, environmental, ethical, and economic benefits (Fehér et al., 2020). Although, the impact of plant-based proteins on EIMD is uncertain. Three studies considered the impact of plant- versus animal- based proteins on resistance EIMD in untrained males in the present review (Hasegawa et al., 2014; Nieman et al., 2020; Saracino et al., 2020). Water or 20 g of egg white or isolated

soy protein was provided to participants by Hasegawa and colleagues (2014) preceding whole-body resistance exercise. Serum [CK] and muscle soreness significantly increased 30 min and 24 h post-exercise, respectively, with no between-group differences. It was found by Nieman et al. (2020) that ~24 g of isolated whey, but not pea, protein consumed before and after whole-body exercise and pre-sleep for 4 d attenuated serum [CK] elevations at 72–96 h post-exercise compared to water consumption. Nonetheless, relative to water, neither protein source reduced muscle strength, endurance, and power decrements nor muscle soreness. Here, the ineffectiveness of plant-based proteins for reducing EIMD might be attributed to their single-source origin. Plant-based proteins, including soy, rice, and wheat, have been scrutinised as inferior in quality to animal-based proteins, due to their lower essential amino acid content (Gorissen et al., 2018) and bioavailability (van Vliet et al., 2015). Ingesting a larger dose (Gorissen et al., 2016) or a blend (Kouw et al., 2021; Pinckaers et al., 2022) of plant-based proteins provides the amino acid profile required to stimulate increased MPS rates. In this review, only one study (Saracino et al., 2020) compared the effect of a plant-based protein blend on EIMD with whey protein isolate, whey protein hydrolysate, and a non-isoenergetic control. EIMD was unaffected by peri-exercise ingestion of a 25–40 g protein dose, irrespective of source, which was perhaps partly due to the equivalent daily total protein intakes between groups. Further investigation of plant- versus animal- based proteins and single-source versus blended plant-based proteins from isolated and whole-food sources is needed to determine the relevance of protein quality in EIMD.

The present findings on the efficacy of ingested protein for muscle function restoration following resistance exercise are consistent with Davies and colleagues (2018). This meta-analysis (n = 13 trials) reported small-medium beneficial effects of whey protein consumption <24–96 h post-exercise. However, peak isometric knee extensor strength was the only outcome considered, and without corroboration from other EIMD markers, these data have narrow application. Further, varied control groups were included (water, carbohydrate, milk, and collagen proteins), making inter-trial generalisability unreasonable. Conversely, the impact of a range of protein- and amino acid- based supplements on EIMD outcomes (muscle function, soreness, CK, LDH, Mb) for up to several weeks following endurance or resistance exercise was examined in the systematic review by Pasiakos et al. (2014). Minimal evidence supporting a benefit of protein supplementation for post-exercise recovery of muscle function and soreness was found by these authors; however, they acknowledge that divergencies in study design regarding protein supplementation and exercise protocols limit their observations. In contrast,

an overall advantage of consuming protein for muscle function was identified in the present review, which may reflect the tighter study inclusion criteria (resistance exercise only, separation of muscle functional markers, exclusion of amino acid-based supplements). Seemingly, broad criteria for study inclusion may mask beneficial effects of protein supplementation for EIMD, especially when small sample sizes prevent sub-group analysis.

3.5.1. Limitations

Several limitations may have affected the outcomes and application of the present review. From the pool of studies, five were considered low/fair quality and therefore susceptible to bias, which can exaggerate treatment effects (Pildal et al., 2007). The main PEDro criteria that studies failed to meet were lack of double blinding, which can induce bias; completion of ≥1 outcome by 85% of participants; and receipt of allocated treatment. However, failure to meet these criteria was often assumed, due to a lack of methods reporting. Other limitations arose from the supplementation strategies and study designs employed. Control supplements were varied (artificial sweetener, water, flavoured water, electrolytes, glucose, maltodextrin, dextrose, and no supplement (Hirose et al., 2013; Karakus & Akkurt, 2020; Kim et al., 2017; West et al., 2017)), and mostly, not isoenergetic to protein supplements; thus, energy/carbohydrate content may have confounded protein effects. A range of protein doses were given, potentially increasing heterogeneity of the study pool. Two studies (Cooke et al., 2010; Kim et al., 2017) prescribed protein dose relative to body mass (1.5 g•kgBM^{-1}) resulting in large doses (~104 g), although most studies provided a standard dose (17–42 g).

Eight studies were possibly limited by their adoption of crossover designs. Due to RBEs associated with EIMD (Fridén et al., 1983; Hough, 1902; Schwane & Armstrong, 1983), responses to repeated exercise were likely attenuated, particularly in untrained participants (Burnley et al., 2010; Hasegawa et al., 2014; Hirose et al., 2013) and with insufficient washout periods (1–2 wk) (Bird et al., 2013; Burnley et al., 2010; Grubic et al., 2019; Hasegawa et al., 2014; Naclerio et al., 2020; West et al., 2017). Notwithstanding, all crossover studies counterbalanced treatment order, which should limit order effects and the impact of RBEs.

Regarding the meta-analyses, ESs were not obtained for all variables in each trial due to insufficient data reporting. However, no apparent differences existed in the outcomes of included or excluded trials. Data extraction from figures may have been inaccurate, resulting in over/underestimated treatment effects. Furthermore, when sample size was not reported for each variable and time-point, a consistent sample size was assumed, which if inaccurate could

have altered true effects. Variables with different assessment methods (e.g. active and passive soreness) were pooled to maximise *k* for meta-analyses; however, this might have impacted overall treatment effects. This review considered only four variables and hence provided scope for future meta-analyses to examine protein supplementation effects on other markers of EIMD. Moreso, due to its large-scale, amino acid-based supplements were not considered in this review, which may have offered beneficial sub-analysis. Finally, as the study samples were 94% young male, the outcomes of this review may be inapplicable to older adults and females.

3.5.2. Future Directions

The limited understanding of the impact of protein supplementation for resistance EIMD management in females should be addressed by conducting high-quality research with females or both sexes. Additional investigation of various protein types (particularly plant-based), timing, and dosing strategies would help inform protein nutrition guidelines for EIMD management. Establishing optimal methods for assessing EIMD in experimental models requires investigation, as methodological inconsistencies across current studies are hindering knowledge progression of EIMD mechanisms and management strategies. To benefit future research, standardised methodologies (e.g. consistent measures, measurement time-points, assessment tools) should be practiced, increasing the generalisability and application of outcomes and data inclusion in topical reviews and meta-analyses. Where feasible, cross-over designs with sufficient wash-out period and, when relevant, unilateral limb models should be employed to limit heterogeneity, although when dietary interventions are concerned, parallel designs may be appropriate. Furthermore, data reporting and transparency issues are limiting study inclusion in meta-analyses and obstructing accurate and representative conclusions being drawn. Accordingly, a framework is proposed outlining data reporting guidance to increase inclusion of primary data in meta-analyses (*Table 3.3*).

3.5.3. Conclusions

This systematic review with meta-analysis aimed to ascertain what the current literature suggests about the overall impact of dietary protein on resistance exercise-induced muscle damage. It was demonstrated by this study that peri-exercise protein consumed by young males reduced maximal strength decrements and lowered [CK] following acute resistance exercise but did not benefit muscle soreness. These outcomes were seemingly unaffected by the type, timing, frequency, and dose of ingested protein, though may have been affected by the exercise protocol and sample training status, with further examination required. An absence of female-

focussed research and a limited number of studies examining plant-based protein sources was identified in this review, which warrant future research priority. Developing evidence-based EIMD management strategies is impeded by methodological inconsistencies across studies, particularly pertaining to EIMD assessment methods. The need for standardised and transparent data reporting in EIMD research was highlighted in this review and a guiding framework has been proposed. Overall, several contributions to knowledge and practical applications were made by this study: dietary protein was indicated to be an effective management strategy for resistance EIMD in males, and therefore, should be consumed peri-exercise to enhance recovery; data from females are lacking, and so, future research should prioritise the inclusion of female participants; and methodological inconsistencies limit the ability to achieve consensus regarding EIMD management strategies and hence, future studies should adopt standardised methods where feasible.

Table 3.3. A framework of data reporting guidelines for primary research to increase inclusion in meta-analyses

Proposed guidelines for data reporting in primary research
Authors should provide supplementary data files of all gathered data where feasible
When measures are conducted at multiple time-points, authors should strive to report the mean change and variance between time-points (not just mean and variance data at each time-point)
Where feasible, baseline data for all measures should be obtained and reported
The acquired sample size for each variable at each time-point should be reported (these data could be included in figure legends or supplementary files)
Studies conducted with participants of both sexes should report male and female data separately (even if the analysis of sex differences is not an outcome of the study)
Authors should report the methods used relating to study quality, e.g. randomisation, blinding, allocation concealment. If no consideration has been given to these methodological factors, this should be stated.

3.6.0. Supplementary Materials

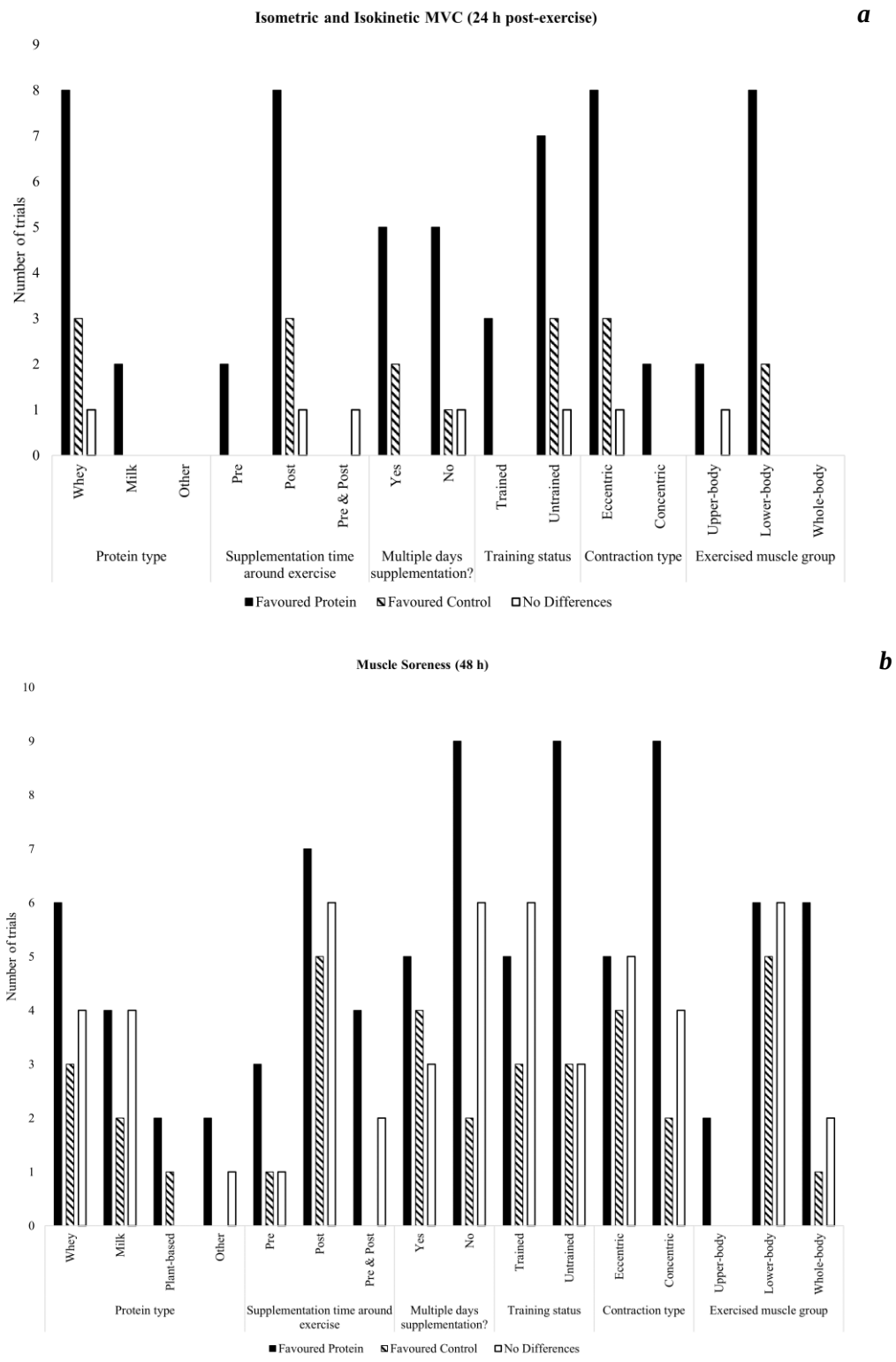


Figure S3.1. Frequency of trials reporting a favourable effect of protein or control supplementation on a. isometric and isokinetic maximal voluntary contraction at 24 h post-exercise and b. muscle soreness at 48 h post-exercise categorised by study variables.

Table S3.1. Magnitude of exercise-induced muscle damage

Reference	Supplement group	Peak change from baseline (%)	EIMD magnitude	Hedges' *g* effect size
Isometric MVC				
Buckley et al. (2010)	Protein (a)	-19.3 ± 8.6	Mild	0.05 [-0.06, 0.15]
	Protein (b)	-30.2 ± 8.8	Moderate	-0.53 [-0.84, -0.23]
	Control	-20.2 ± 23.7	Moderate	
Cooke et al. (2010)	Protein	-12.5 ± 13.8	Mild	0.64 [0.26, 1.02]
	Control	-21.0 ± 11.0	Moderate	
Dale et al. (2015)	Protein (a)	-22.4 ± 4.9	Moderate	-0.42 [-0.65, -0.19]
	Protein (b)	-23.5 ± 3.4	Moderate	-0.82 [-1.24, -0.40]
	Control	-20.4 ± 3.8	Moderate	
Draganidis et al. (2017)	Protein	-27.4*	Moderate	0.91 [0.51, 1.31]
	Control	-39.6*	Moderate	
Farup et al. (2014)	Protein	-24.2 ± 18.7	Moderate	0.27 [0.06, 0.48]
	Control	-28.7 ± 12.8	Moderate	
Ives et al. (2017)	Protein	-22.1 ± 10.4	Moderate	0.25 [0.07, 0.43]
	Control	-25.8 ± 17.6	Moderate	
Kim et al. (2017)	Protein (a)	-56.7 ± 15.1	Severe	0.31 [0.04, 0.58]
	Protein (b)	-59.7 ± 20.0	Severe	0.11 [-0.05, 0.28]
	Protein (c)	-60.8 ± 18.8	Severe	0.06 [-0.06, 0.18]
	Control	-62.2 ± 12.4	Severe	
Isokinetic MVC				
Cockburn et al. (2010)	Protein (a)	-22.0 ± 14.0	Moderate	0.06 [-0.06, 0.19]
	Protein (b)	-7.0 ± 30.0	Mild	0.46 [0.13, 0.80]
	Protein (c)	-16.0 ± 24.0	Mild	0.3 [0.03, 0.57]
	Control	-27.0 ± 42.0	Moderate	
Cockburn et al. (2012)	Protein (a)	-14.0 ± 15.5	Mild	0.13 [-0.05, 0.31]
	Protein (b)	-12.5 ± 14.0	Mild	0.2 [-0.02, 0.42]
	Control	-17.0 ± 26.5	Mild	
Cooke et al. (2010)	Protein	-8.6 ± 17.7	Mild	0.37 [0.08, 0.66]
	Control	-14.0 ± 7.1	Mild	
Philpott et al. (2018)	Protein	-9.7 ± 9.6	Mild	1.34 [0.84, 1.85]
	Control	-30.0 ± 5.8	Moderate	
Rankin et al. (2015)	Protein	-11.4 ± 25.4	Mild	0.7 [0.41, 0.98]
	Control	-29.1 ± 24.2	Moderate	

*The magnitude of exercise-induced muscle damage (EIMD) is based on the peak percent reduction from baseline in isometric (k = 11) and isokinetic (k = 8) maximal voluntary contraction (MVC) and categorised as mild (<20% MVC reduction), moderate (20–50% MVC reduction), or severe (>50% MVC reduction) as per Paulsen et al. [54]. Only trials included in the meta-analyses are presented alongside the Hedges' g effect size at the time-point of peak MVC change; data expressed as mean ± standard deviation (SD); *SD not reported in manuscript (g calculated from reported ES).*

3.7.0. Link to Next Chapter

A near absence of sex-comparative and female-focussed research on the impact of protein supplementation on resistance EIMD was identified by this systematic review, and to this end, an RCT including both males and females was conducted in this thesis in attempt to address this gap. It was also highlighted by this systematic review that most experimental EIMD protocols have been extreme and unrealistic of habitual training practices. It was deemed important for this thesis to investigate the magnitude of muscle damage that would be encountered by 'real-world' exercisers and whether the response to protein ingestion differed to when severe EIMD was induced. Furthermore, a distinct influence of protein type on muscle damage responses was not found by the meta-analysis, and given that current sport nutrition guidelines advocate a food-first approach, this thesis wanted to examine the effect of a whole-food dietary staple protein source on EIMD.

CHAPTER 4 Milk Protein Ingestion does not Enhance Recovery from Muscle-Damaging Resistance Exercise in Untrained Males and Females: A Randomised Controlled Trial

4.1.0. Prelude

This thesis aimed to establish a convenient and easily accessible EIMD recovery strategy for novice exercisers in an attempt to increase long-term adherence to exercise training that is needed for chronic muscle adaptation. It was considered important that the dietary intervention be not too far removed from habitual dietary practices, by involving a commonly consumed dairy product – again, with the rationale of promoting adherence. Many previous studies of this nature have involved isolated protein sources, and when applying these outcomes to real-world settings, may lack appeal to those unfamiliar with consuming dietary supplements, particularly in powder or gel form. Further, it was hypothesised that sex differences in EIMD responses would be apparent and to this end, males and females were recruited for the RCT. Determining whether sex differences also exist in EIMD responses to protein ingestion by sub-grouping males and females within the supplement conditions was considered valuable.

Moreover, in attempt to ascertain the potential factors governing sex differences in EIMD, this chapter considers the interplay between body composition and muscle damage. Importantly, regional as well as total body composition outcomes were analysed for associations with peak changes in EIMD markers, as it was hypothesised that greater muscle mass of the exercised limb may equate to greater muscle damage. Given that males tend to possess greater muscle mass than females, this hypothesis was tested to help explain the sex differences identified within this chapter. Accordingly, correlational analyses were conducted separately for males and females.

A modified version of this chapter has been published in Applied Physiology, Nutrition, and Metabolism.

4.2.0. Introduction

Milk-based foods provide a rich source of carbohydrate, micronutrients, and a complete amino acid profile, and therefore, may optimise exercise recovery (James et al., 2019). Cow's milk stimulates comparable rates of muscle glycogen resynthesis (Ferguson-Stegall et al., 2011) and rehydration (Seery & Jakeman, 2016) following cycling exercise compared with carbohydrate ingestion. Due to its essential amino acid content, particularly leucine (Rafiq et al., 2016), milk protein has been a common choice of post-exercise nutrition to stimulate MPS (Wilkinson et al., 2007). The amount of protein required to maximally stimulate MPS rates after leg-based exercise (20 g) (Moore et al., 2009; Witard et al., 2014) can be conveniently achieved with the ingestion of ~555 mL of cow's milk or as little as 170 g of dairy yoghurt. By frequently elevating MPS rates, regular milk protein consumption alongside resistance training can promote exercise training adaptations (Hartman et al., 2007; Josse et al., 2010). Therefore, milk protein may offer an ideal nutritional aid to optimise several domains of post-exercise recovery.

One exercise recovery component that may be modulated by milk protein consumption is EIMD. Exercise-induced muscle damage is a consequence of unaccustomed or eccentric muscle contractions (Stauber, 1989) and is therefore a particular risk to individuals naïve to resistance exercise. Exercise-induced muscle damage is characterised by a temporary reduction in skeletal muscle functional capacity; the release of intramuscular proteins into the circulation; and perceived feelings of muscle soreness (Allen, 2001; Clarkson & Hubal, 2002; Morgan et al., 1996; Pyne, 1994; Warren et al., 1999), which may prolong exercise recovery and limit future training quality. Acute, severe muscle damage can hinder chronic muscle adaptations to exercise (Damas et al., 2016$_b$; Eriksson et al., 2006; Foley et al., 1999; Lauritzen et al., 2009), although, whether mild muscle damage is a prerequisite for exercise adaptation by preparing the muscle for future hypertrophy warrants investigation (Damas et al., 2018). Experimental models of EIMD typically incorporate extreme exercise stimuli to maximise damage and do not reflect the magnitude of muscle damage that occurs during habitual training (i.e. milder). Therefore, examination of mild muscle damage induced by physiologically relevant exercise protocols is warranted.

The impact of ingested milk protein on the management of EIMD is equivocal. Following unilateral leg flexions with post-exercise consumption of cow's milk, proving 34 g of protein, markers of EIMD were assessed in trained males by Cockburn and colleagues. The consumption of cow's milk attenuated maximal strength decrements and serum [CK]

elevations compared with post-exercise carbohydrate ingestion (Cockburn et al., 2008). Subsequent studies demonstrated that consuming half the quantity of cow's milk (17 g of protein) was sufficient to attenuate symptoms of EIMD (Cockburn et al., 2012; 2013). While supporting data have been provided (Draganidis et al., 2017; Norikazu et al., 2013), others reported no differences in muscle soreness, MVC, or serum CK with the ingestion of milk protein versus carbohydrate beverages (Gee et al., 2019; Rankin et al., 2015; Wojcik et al., 2001). Further, the influence of milk protein ingestion timing in relation to exercise is unclear (Cockburn et al., 2010). Therefore, the optimal amount, timing, and source of milk protein to ingest in relation to exercise for the management of EIMD is currently uncertain.

Most research has been conducted with males, despite several reports of sex differences in EIMD (Fernandez-Gonzalo et al., 2014; Fredsted et al., 2008; Kerksick et al., 2008; Minahan et al., 2015; Sewright et al., 2008; Stupka et al., 2000). Only one study has examined EIMD responses to milk protein ingested by trained, young adults of both sexes (Rankin et al., 2015). Cow's milk was ingested following maximal eccentric knee flexion exercise and compared with an isoenergetic carbohydrate beverage. A beneficial effect of milk consumption on the change in peak torque and passive muscle soreness up to 72 h post-exercise was experienced by females, while it was unclear whether milk consumption was beneficial in males, indicating a sex-dependent response. Evidently, data extrapolated from males is not commensurate with females, and therefore, further research conducted with both sexes into the management of EIMD is warranted.

One factor that may be implicated in the disparate EIMD responses between sexes is body composition. Body composition, notably skeletal muscle mass, displays marked differences between males and females (Abe et al., 2020; Kutáč & Sigmund, 2017; Miller et al., 1993; Santos et al., 2013) and has been investigated in an attempt to explain the inter-individual variability and contributing factors of EIMD (Bekkelund & Jorde, 2018; Comstock et al., 2013; Yoon & Kim, 2020). Individuals with greater muscle mass, particularly males compared with females, have experienced heightened serum [CK] following damaging exercise (Hicks et al., 2016; Minahan et al., 2015; Sewright et al., 2008). Considering serum [CK] predominantly reflects CK efflux from skeletal muscle (Baltusnikas et al., 2015), it may be speculated whether the higher [CK] in males than females is due to their greater muscle mass. At rest, serum [CK] is poorly associated with absolute muscle mass in both sexes (Bekkelund & Jorde, 2018; Norton et al., 1985; Novak & Tillery, 1977; Swaminathan et al., 1988). However, the impact of muscle mass on [CK] following exercise is not well understood, largely because most studies

have used invalid estimations of muscle mass, e.g. skinfold thickness measurements. Studies using more valid assessment methods (DXA, BIA, and air displacement plethysmography) have found no association between muscle mass and post-exercise [CK] or other markers of EIMD in trained (Fernandes et al., 2020) or untrained (Kim & Lee, 2015) males. Nonetheless, owing to sex-specific EIMD responses (Fredsted et al., 2008; Hicks et al., 2016; Kerksick et al., 2008; Minahan et al., 2015; Sewright et al., 2008), the relationship of muscle mass and EIMD may differ in females, which has not yet been investigated.

Adipose tissue may also play a role in EIMD responses. Overweight males and females have appeared more susceptible to EIMD than healthy weight individuals (Kim & So, 2018; Paschalis et al., 2010; 2013), which has been attributed to excess adiposity (Hickner et al., 2001; Kim & Lee, 2015). Excess body fat or dietary fat overconsumption can increase the saturation of fatty acyl chains within the sarcolemma, which in turn increases phospholipid packing density (Andersson et al., 2002; Hazel & Williams, 1990). As a consequence, myofibre membranes become too rigid, rendering them weaker and more vulnerable to damage (Knoblauch et al., 2013). Exercise-induced membrane damage increases intramuscular CK release into circulation, which has shown to be greater in males and females with higher compared to lower BF% (Heled et al., 2007; Kim & Lee, 2015; Margaritelis et al., 2019; Paschalis et al., 2010; 2013). Furthermore, adipose tissues are the source of various pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 (Fonseca-Alaniz et al., 2007), which have been implicated in EIMD (Konrad et al., 2011; Paulsen et al., 2012; Pyne, 1994; Zhang et al., 2020). Exercise-induced local inflammation is a probable contributor to muscle swelling and soreness (Nosaka & Clarkson, 1996$_a$; Philpott et al., 2018), which can be increased to a greater extent in overfat relative to lean males and females following eccentric exercise (Kim & Lee, 2015; Margaritelis et al., 2019; Paschalis et al., 2010; 2013). Therefore, individuals with higher BF% may experience augmented post-exercise inflammation and myofibre membrane damage, contributing to symptoms of EIMD.

Myofibre damage occurs exclusively to the exercised muscle (Armstrong et al., 1983; Carpenter & Karpati, 1989; Ogilvie et al., 1988) and hence the relationship of regional body composition and EIMD is of interest. While some markers of EIMD, such as blood biomarkers, are obtained systemically, most are obtained locally from the site of injury, i.e. the exercised limb, including measures of flexibility, strength, swelling, and soreness. Therefore, the body composition of the exercised region may impact these local EIMD markers. To this end, midthigh lean tissue area was estimated from measures of leg circumference and skinfold

thickness, and body fat was calculated from measures of residual volume and body density by Hickner et al. (2001). These authors reported a positive association between the fat mass-to-midthigh lean tissue area ratio and the decline in maximal strength, but not muscle soreness nor [CK], 48 h following downhill running exercise performed by trained males. Hence, indicating that local EIMD markers may be impacted by the body composition of the exercised region. However, this relationship has yet to be explored in females and untrained males using more direct, valid assessments of regional body composition.

To address these knowledge gaps and help bridge the sex data gap, the present study held three aims: (1) to investigate the impact of milk protein ingestion on recovery from muscle damaging resistance exercise, (2) to ascertain whether EIMD responses differ between untrained males and females, and (3) to explore the relationship of EIMD and DXA-derived total and regional body composition.

4.3.0. Methods

4.3.1. Experimental Design

A randomised, single-blind, parallel group trial examined the impact of milk protein ingestion on indirect markers of muscle damage during 7 days of post-exercise recovery. Following initial eligibility screening, participants attended two familiarisation sessions to assess maximal leg strength and body composition. Participants were then equally randomised by drawing a folded piece of paper labelled 'A' or 'B' from an opaque envelope to a milk protein (MILK-PRO: Female-PRO, n = 8; Male-PRO, n = 4) or control (CON: Female-CON, n = 8; Male-CON, n = 4) group. A three-week period separated the familiarisation and muscle damage exercise sessions to reduce the influence of RBEs and standardise MC phase in females. Participants consumed one dose of their allocated supplement pre-exercise and 16 doses over 5 days post-exercise. Venous blood samples and measures of limb circumference, ROM, and muscle soreness were obtained pre, immediately post, and 24, 48, 72, and 168 h post-exercise. Maximal leg strength was measured at +72 and +168 h. All study procedures were conducted in the Human Performance Laboratory, Truscott Imaging Suite, and Fitness Centre at Durham University. The study was approved by the Tyne and Wear South NHS Research Ethics Committee (21/NE/0073) and the Department of Sport and Exercise Sciences Research Ethics Sub-Committee, Durham University, and all participants provided signed informed consent. This trial was prospectively registered at ClinicalTrials.gov PRS (protocol ID: 290580A). The study design is presented in *Figure 4.1*.

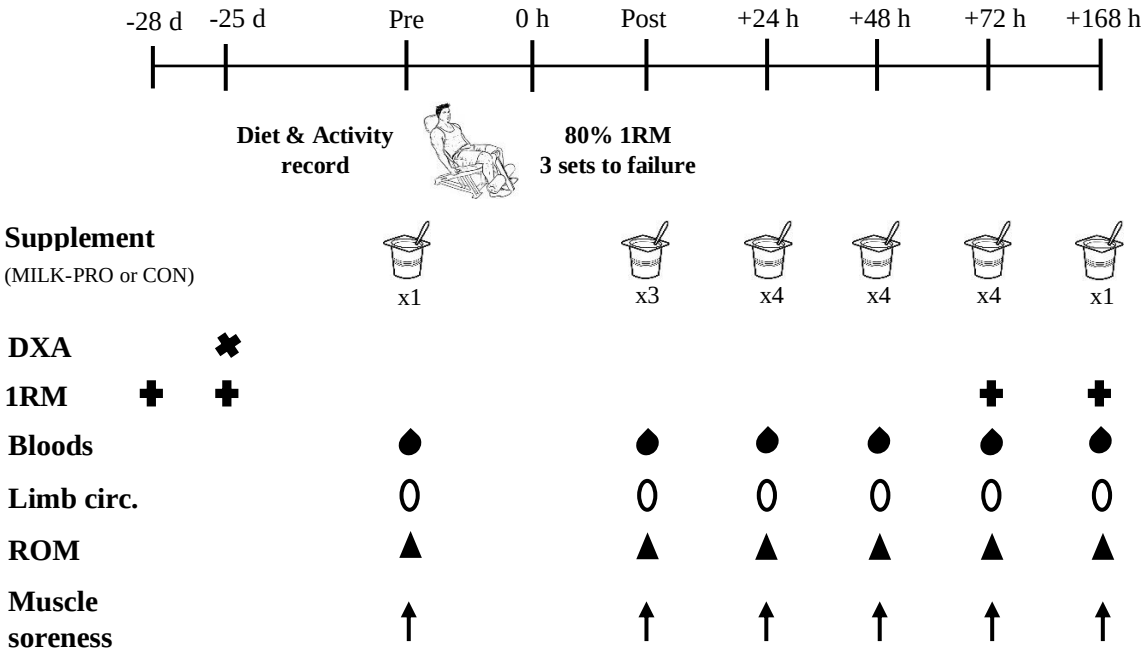


Figure 4.1. Study design.
DXA = dual-energy X-ray absorptiometry; 1RM = one repetition maximum; limb circ. = limb circumference; ROM = range of motion. MILK-PRO = milk-protein supplement (milk-based yoghurt) providing 20 g of protein; CON = control supplement (isoenergetic oat-based yoghurt).

4.3.2. Participants

A statistical power analysis was conducted using G*Power 3.1 to determine the study sample size. The power calculation was based on a similar study by Cockburn et al. (2008), which reported a significant difference in the change in isokinetic MVC from baseline between milk protein and carbohydrate groups of 25%. The calculation revealed that 5 participants per group (20 total) were required to have 80% power to detect significant between-group differences when using a dependent *t*-test with 0.05 two-sided significance level. Therefore, 24 participants were required to allow for 20% dropout.

Twenty-five healthy, untrained participants volunteered and were eligible for this study, and 24 completed the study (females: n = 16; age 23.9 ± 4.7 y; 44% White, 44% Asian, 12% other, males: n = 8; age 26.1 ± 5.6 y; 63% White, 37% other). Two participants failed to attend one laboratory visit (+168 h) due to illness. An additional 6 participants (n = 4 female, n = 2 male; age 27.3 ± 5.0 y) volunteered to receive repeat body composition assessments and were included in the DXA precision error assessment. Participants met the following inclusion criteria: free from musculoskeletal disorders and injury; do not habitually (twice per week for previous one-month period) consume nutritional supplements, ergogenic aids, or non-steroidal anti-inflammatory drugs; do not frequently engage in therapies that may alleviate muscle damage (e.g. massage, cryotherapy); and have not performed resistance or eccentric exercise during the previous 6 months. Female participants were naturally menstruating (self-reported regular MC during the previous 12 months) or used HCs (n = 2 MILK-PRO, n = 2 CON: combined pill, progesterone-only pill, or Depo-Provera injection) and not pregnant.

4.3.3. Baseline Assessments and Familiarisation

Participants completed an online health and readiness to exercise questionnaire. For naturally menstruating females, all baseline and experimental measurements were conducted during the late follicular phase of the MC (days 5–11). Cycle phase was estimated from self-reported data on timing and duration of menses, and sessions were conducted as close as feasible following the last day of menses. For males and female users of hormonal contraceptives, measurements

were conducted at any time due to the inability to estimate pseudo cycle phase (i.e. due to absence of withdrawal bleed).

Maximal strength was assessed with a 1RM test (Baechle & Earle, 2008) at -28 d, which served as a familiarisation session, and repeated at -25 d to confirm 1RM (McCurdy et al., 2004; Ritti-Dias et al., 2011). Participants were demonstrated the correct form for using the leg extension and leg curl exercise machines (Versa leg extension/leg curl, Matrix, Wisconsin, USA) before completing a warm-up set with a light load (10 repetitions, easily performed). The exercise load was progressively increased by 10–20% for each successive single full repetition attempt , with a 3 min inter-set rest period. Following a failed attempt, the exercise load was reduced by 5–10% until 1RM was established. This protocol was completed using the leg extension and then leg curl machine with 5 min rest between exercises. The test-retest reliability of the 1RM protocol has been reported as good to excellent [median intra-class correlation coefficient (ICC) = 0.97; CV = 4.2%], independent of sex, age, and training experience (Grgic et al., 2020).

Body composition was assessed using DXA (Lunar iDXA, GE Healthcare, Madison, WI) at -25 d. Participants followed a standardised pre-scan protocol (Hind et al., 2018; Nana et al., 2015) and were measured wearing minimal, metal-free clothing (e.g. t-shirt and shorts) and with jewellery removed. Body mass was measured to the nearest 100 g and stature to the nearest mm (Seca Weighting and Measuring Systems, Birmingham, UK). Participants were positioned centrally and supine on the DXA scan bed with hands in a mid-prone position and head in the frankfort plane. Two scans were completed after re-positioning to enable calculation of precision error. Total body mass, LBM, fat mass, BF%, and bone mineral content (BMC) were derived. Precision error [coefficient of variation (CV%) (Root Mean Square SD)] was 0.98% (0.152 g•cm^{-2}), 0.37% (0.162 g•cm^{-2}), and 0.41% (0.169 g•cm^{-2}) for the assessments of fat mass, FFM, and LBM, respectively. Regional body composition was analysed by manually selecting the region of interest (exercised limb; upper-leg) using the DXA software system (enCORE V.18, GE Healthcare). The segmental lines were placed at the lower and upper ends of the trochanterion-tibiale lateral site as per *Figure 4.2* to encompass the quadricep and hamstring muscles and surrounding fat tissue. Manually selecting regions of interest for lower extremities is highly reliable (r = 0.95–0.98) (Lohman et al., 2009).

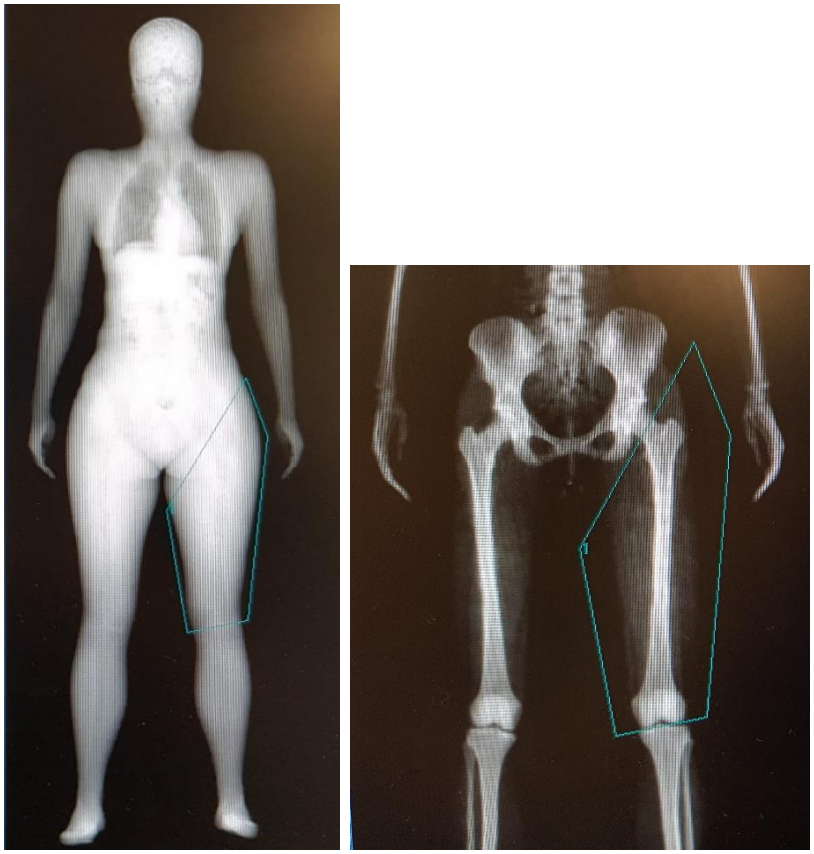


Figure 4.2. DXA scan output depicting the region of interest for the analysis of regional body composition.

4.3.4. Physical Activity and Dietary Control

Participants recorded habitual activity and dietary intake for 3 random days during the 3-week wash-out period (baseline dietary intake). Three hours before attending the laboratory for the experimental trials (+0–72 and +168 h), participants were guided to consume a standardised breakfast (238 kcal, 8 g protein, 45 g carbohydrate, 3 g fat) alongside their supplement. Besides the prescribed breakfast and supplement, participants consumed their habitual diet, which was assessed with 24-hour dietary recalls at each visit. Participants were instructed to abstain from the use of non-steroidal anti-inflammatory drugs, strenuous exercise, engagement in massage or cryotherapy, and the consumption of alcohol, additional protein supplements to those provided, vitamin and mineral supplements, and ergogenic aids during the study period. Dietary intake was analysed using Nutritics software (Nutritics, Dublin, Republic of Ireland).

4.3.5. Supplementation

The experimental supplement was a milk-protein based yoghurt (MILK-PRO; Arla Foods Ltd, Skyr Icelandic style yoghurt, strawberry) and the control intervention was an isoenergetic oat-based yoghurt (CON; Oatly AB, Oatgurt, strawberry) (*Table 4.1*). Participants consumed 4 doses per day of their allocated supplement at ~4 h intervals during +0–72 h and one dose on the morning of +168 h (17 doses in total). Supplements were indistinguishable in taste, texture, and appearance and were provided by the lead researcher in plastic pots labelled 'A' or 'B' to ensure participant blinding.

Table 4.1. Nutritional composition of the yoghurt supplements. MILK-PRO = milk-protein supplement (milk-based yoghurt); CON = control supplement (isoenergetic oat-based yoghurt)

	MILK-PRO		CON	
	Per 100 g	*Per dose (208 g)*	*Per 100 g*	*Per dose (150 g)*
Energy (Kcal)	73	152	101	152
Protein (g)	9.6	20	1.4	2.1
Carbohydrate (g)	7.4	15.4	16	24.1
Fat (g)	<0.5	<1	3.2	4.8

4.3.6. Resistance Exercise

Resistance exercise sessions were supervised. The protocol was performed on the leg extension followed by the leg curl machine, separated by 5 min rest. Participants performed a warm-up of 10 repetitions at 50% of their pre-determined 1RM. Three sets of each exercise (2 min inter-set rest) were completed at 80% 1RM to volitional failure. Lifting tempo was targeted as 1 and 2 s for concentric and eccentric phases of muscle contraction, respectively, which naturally slowed as muscle fatigue increased. Strong verbal encouragement was given to all participants during exercise.

4.3.7. Muscle Damage Markers

Participants attended the laboratory at approximately the same time each day. The following assessments were conducted in the same order during each visit (pre, post, +24, +48, +72, +168 h). Blood samples were collected from an antecubital vein of the forearm using standard venepuncture techniques into three reagent-free vacutainers (10 mL). Limb circumference was measured using a standard anthropometric measuring tape at the mid-, lower-, and upper-quartile points of the trochanterion-tibiale lateral site with the participant in a standing position. The mean value of these three sites was used for analysis. Knee joint ROM was calculated as the difference between the relaxed and flexed knee joint angle, as measured using a standard

goniometer with the participant supine. Muscle soreness was rated separately for the quadricep, hamstring, glute, and calf muscles using a 10-point VRS ranging from '0 – not sore at all' to '10 – extremely sore' while performing a simple bodyweight squat. Mean soreness was calculated from these values. Pressure-pain threshold was assessed using a computerised pressure algometer (Medoc, AlgoMed, Ramat Yishai, Israel) with the participant supine. The probe head (1 cm^2) of the algometer was placed at the mid-, lower-, and upper- quartile points of the trochanterion-tibiale lateral site and increasing pressure was applied until the participant verbally indicated pain. The mean value of these three sites was used for analysis. At +72 and +168 h, the 1RM test was repeated. One-repetition maximum was not assessed at 0–48 h as it would not align with habitual training practices to perform a maximal strength test between exercise bouts, and it is advised that untrained individuals perform resistance exercise 2–3 $d•wk^{-1}$ (i.e. ~2 d rest between sessions) (ACSM, 2009).

4.3.8. Serum Preparation and Analysis

Whole-blood samples were left at room temperature for 30 min before being stored on ice (maximum 2 h) and then centrifuged at 4 °C with 1100 g force for 15 min. Serum samples were transferred into 1 mL microcentrifuge tubes and stored at -80 °C until analysis. Diluted samples (×200 dilution factor) were measured in triplicate for [CK] using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abcam plc, Cambridge, UK) and sample optical density was measured using a microplate reader at a wavelength of 450 nm. Any outliers within the triplicate measures were excluded prior to calculating the mean of the values for statistical analyses. The intra-assay precision of this ELISA was 20.7%.

4.3.9. Statistical Analyses

Statistical analyses were conducted using IBM SPSS (version 25, SPSS Inc., Chicago, IL). Shapiro–Wilk and Levene's tests were used to assess statistical assumptions and equality of variances between groups, respectively. Data that violated the assumptions were analysed with the equivalent non-parametric test (non-parametric variables: exercise, 1RM, VRS, and CK). Data were analysed with mixed analysis of variance/Kruskal Wallis and Friedman's tests and data that violated the Mauchly's test of sphericity were corrected with Huynh–Feldt. Any significant interactions were analysed using independent *t*-tests/Mann–Whitney U for between-group comparisons and paired *t*-tests/Wilcoxon signed-rank for within-group comparison. Bonferroni corrections were used to correct for multiple comparisons. To examine sex differences, MILK-PRO and CON data were pooled, and data were examined for differences

between males and females with Mann–Whitney U tests/independent samples *t*-tests. Paired *t*-tests/Wilcoxon signed ranks tests were used to assess the significance of pre-peak changes (calculated as the group mean of the greatest change from pre-exercise for each participant individually) in EIMD markers. Hedges' *g* ESs with CIs were calculated using the standardised mean difference between males and females in the change from baseline to each post-exercise time-point for EIMD markers. Outcomes of total and regional body composition were independently related to the pre-peak change in each marker of EIMD using Spearman's *Rho* (r_s) correlation coefficient. Interpretation of the correlation coefficient was as follows: 0.00–0.19 = very low association; 0.20–0.39 = low association; 0.40–0.69 = modest association; 0.70–0.89 = high association; 0.90–1.00 = very high association. Statistical significance was set at $p < 0.05$. Confidence intervals assume 95% confidence in the range of the mean. Data are reported as mean ± SD unless otherwise stated.

4.4.0. Results

All participants received the supplement they were allocated at baseline (59% failed to identify their supplement) and reportedly consumed all doses. Baseline body composition data are presented in *Table 4.2*. There were no significant differences in body composition between MILK-PRO and CON overall, although male-CON had significantly greater LBM than male-PRO. Males had significantly lower BF% and greater stature, LBM, and BMC than females ($p < 0.01$). There were no significant differences in body composition of the dominant compared with the non-dominant leg in either sex.

Table 4.2. Dual-energy X-ray absorptiometry-derived body composition outcomes

	Females (n = 16)	Males (n = 8)
Total body composition		
Stature (cm)	164.5 ± 3.7‡	181.5 ± 5.3
Body mass (kg)	60.5 ± 10.2†	71.5 ± 11.8
Body mass index (kg•m^{-2})	22.4 ± 3.6	21.7 ± 3.2
Lean body mass (kg)	38.1 ± 3.5‡	53.4 ± 3.8
Fat mass (kg)	20.0 ± 7.2	14.9 ± 8.9
Body fat (%)	33.5 ± 7.3‡	20.6 ± 9.7
Bone mineral content (kg)	2.3 ± 0.3‡	3.0 ± 0.6
Bone mineral density (g•cm^{-2})	1.208 ± 0.102	1.268 ± 0.179
Z-score	1.32 ± 1.22	0.54 ± 1.97
Regional body composition		
Dominant leg		
Mass (kg)	7.5 ± 1.6	8.7 ± 1.8
Lean mass (kg)	4.6 ± 0.6‡	6.7 ± 0.7
Fat mass (kg)	2.8 ± 1.1	1.7 ± 1.2
Fat mass (%)	36.3 ± 7.1‡	18.7 ± 10.2
Bone mineral content (g)	192.8 ± 30.5‡	272.4 ± 59.6
Non-dominant leg		
Mass (kg)	7.6 ± 1.6	8.6 ± 1.8
Lean mass (kg)	4.6 ± 0.6‡	6.6 ± 0.6
Fat mass (kg)	2.8 ± 1.0	1.7 ± 1.2
Fat mass (%)	36.3 ± 7.2‡	18.9 ± 10.2
Bone mineral content (g)	191.5 ± 28.3‡	269.3 ± 57.6

Data are the mean values of two consecutive scans conducted at baseline (-25 d) and are reported as the group mean ± standard deviation.

†Significantly different to males ($p < 0.05$); ‡Significantly different to males ($p < 0.01$)

4.4.1. Dietary Intake

Table 4.3 displays dietary intake data. There were no significant differences between groups in baseline dietary intake except for energy intake (higher in Male-PRO than Female-PRO; p = 0.045). Absolute and relative protein intake increased in MILK-PRO (60.7 g and 1.03 g•d^{-1}•kgBM^{-1}, respectively) equally between sexes and was unchanged in CON. During supplementation, the increase from baseline in absolute, but not relative, carbohydrate intake was significantly greater for CON than MILK-PRO (p = 0.028) with no differences between sexes. There were no significant effects of time or group*time interactions for fat intake.

Table 4.3. Participants' dietary intake

	Female-PRO	Female-CON	Male-PRO	Male-CON	Overall time effect
Baseline: dietary intake record (3 days)					
	(n = 7)	(n = 8)	(n = 3)	(n = 2)	
Energy (kcal)	1772 ± 282	1943 ± 303	2457 ± 529$^{\text{Y}}$	1949 ± 14	
Protein (g)	72.5 ± 24.1	74.6 ± 22.2	109.6 ± 24.0	87.0 ± 0.1	
Protein (g•d^{-1}•kgBM^{-1})	1.3 ± 0.5	1.2 ± 0.4	1.6 ± 0.4	1.3 ± 0.2	
CHO (g)	204.0 ± 21.2	215.0 ± 54.0	302.2 ± 82.8	201.2 ± 30.1	
CHO (g•d^{-1}•kgBM^{-1})	3.5 ± 0.7	3.5 ± 0.9	4.2 ± 0.4	2.9 ± 1.0	
Fat (g)	73.3 ± 20.3	83.1 ± 21.0	89.1 ± 25.1	77.8 ± 3.4	
Fat (g•d^{-1}•kgBM^{-1})	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.1 ± 0.3	
Supplement period: 24 h recall (3 days)					
	(n = 8)	(n = 8)	(n = 4)	(n = 4)	
Energy (kcal)	2164 ± 286	2315 ± 455	2721 ± 466	2686 ± 568	$p < 0.001^{*}$
Protein (g)	135.3 ± 12.7	81.1 ± 13.8$^{‡¥}$	159.7 ± 20.0	91.2 ± 19.8$^{‡¥}$	$p < 0.001^{*}$
Protein (g•d^{-1}•kgBM^{-1})	2.4 ± 0.5	1.3 ± 0.3$^{‡¥}$	2.4 ± 0.2	1.3 ± 0.5$^{‡¥}$	$p = 0.002^{*}$
CHO (g)	249.8 ± 48.1	296.6 ± 59.9	345.3 ± 57.7	364.6 ± 123.2	$p < 0.001^{*}$
CHO (g•d^{-1}•kgBM^{-1})	4.4 ± 0.8	4.8 ± 1.1	5.2 ± 0.9	4.9 ± 2.2	$p < 0.001^{*}$
Fat (g)	64.4 ± 11.8	96.1 ± 27.3$^{\text{Y}}$	77.3 ± 26.8	88.4 ± 9.8	$p = 0.732$
Fat (g•d^{-1}•kgBM^{-1})	1.1 ± 0.3	1.6 ± 0.5	1.2 ± 0.4	1.2 ± 0.2	$p = 0.867$

*Data obtained at baseline from a 3-day self-reported dietary intake record and during the supplementation period from 24-hour dietary recalls Overall time effect refers to the corresponding value at baseline. Data are reported as the group mean ± standard deviation. ‡Significantly different to Male-PRO ($p < 0.01$); ʸSignificantly different to Female-PRO ($p < 0.05$); ¥Significantly different to Female-PRO ($p < 0.01$); *Statistically significant at the level of $p < 0.01$.*

4.4.2. Resistance Exercise

Table 4.4 describes the muscle damage exercise bout. Males performed significantly more repetitions for sets one and two of leg extension than females. Exercise load and volume was higher in males compared with females for both exercises (all $p < 0.05$).

Table 4.4. Muscle damage resistance exercise bout conducted on day 0

	Female-PRO (n = 8)	Female-CON (n = 8)	Male-PRO (n = 4)	Male-CON (n = 4)
Leg extension				
Load (kg)	53.7 ± 10.6[‡§]	48.1 ± 10.4[‡§]	81.1 ± 3.5	82.3 ± 3.5
Set 1 reps	10.8 ± 5.5	12.1 ± 3.7	15.3 ± 1.9	15.0 ± 2.6
Set 2 reps	9.1 ± 4.6	9.6 ± 2.3	11.3 ± 2.2	14.0 ± 1.2
Set 3 reps	8.8 ± 5.1	8.8 ± 3.5	9.5 ± 0.6	10.3 ± 3.1
Volume (kg)	1481.0 ± 624.6[‡§]	1479.8 ± 457.5[‡§]	2911.7 ± 254.7	3242.8 ± 651.3
Leg curl				
Load (kg)	40.7 ± 6.2[‡§]	36.2 ± 8.5[‡§]	64.4 ± 10.0	65.8 ± 10.4
Set 1 reps	16.8 ± 6.9	14.6 ± 4.9	18.0 ± 3.2	17.5 ± 4.8
Set 2 reps	14.6 ± 4.9	12.4 ± 3.7	19.0 ± 8.3	12.8 ± 1.9
Set 3 reps	15.5 ± 8.0	14.4 ± 7.6	17.0 ± 9.0	11.5 ± 3.3
Volume (kg)	1882.4 ± 715.7[†]	1453.3 ± 428.5[‡Ŧ]	3333.9 ± 665.9	2707.2 ± 416.8

Data are reported as the group mean ± standard deviation. [†]Significantly different to Male-PRO ($p < 0.05$); [‡]Significantly different to Male-PRO ($p < 0.01$); [Ŧ]Significantly different to Male-CON ($p < 0.05$); [§]Significantly different to Male-CON ($p < 0.01$).

4.4.3. One-Repetition Maximum

Leg extension: Across all groups, 1RM increased over time (*Figure 4.3*). One-repetition maximum was comparable between MILK-PRO and CON though significantly lower in females than males at all time-points ($p < 0.01$). When normalised to body mass, 1RM remained higher in males than females at -25 d (1.5 ± 0.2 vs 1.1 ± 0.2 kg•kgBM^{-1}; $p = 0.002$) but was comparable at other time-points and when normalised to LBM. Peak changes in 1RM from -25 d were comparable between males and females (-2.8 ± 12.1 and 3.0 ± 4.7 kg, respectively; $p = 0.120$). ES at +72 and +168 h significantly favoured females (*Figure 4.6*).

Leg curl: 1RM equally increased over time between groups (*Figure 4.3, Supplementary table S4.1*). There were no differences between MILK-PRO and CON at any time-point. Absolute 1RM was higher in males than females at all time-points ($p < 0.01$), which remained higher in males at -28 d, +72 h, and +168 h when normalised both to body mass and LBM ($p \leq 0.042$). Peak increases in 1RM from -25 d were comparable between males and females (6.6 ± 9.7 and 3.0 ± 3.4 kg, respectively; $p = 0.070$). Effect sizes at +72 and +168 h significantly favoured males (*Figure 4.6*).

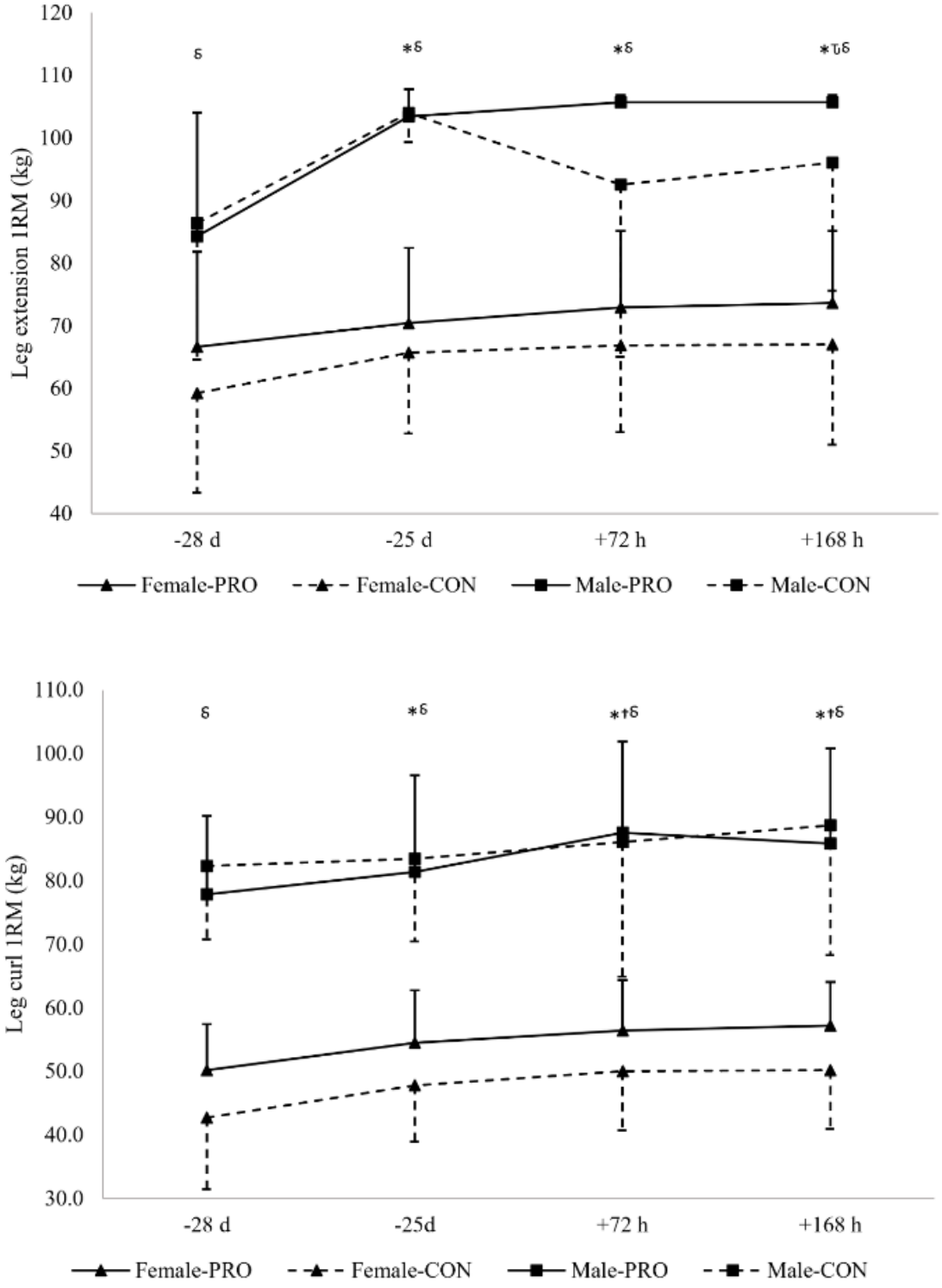


Figure 4.3. Absolute one-repetition maximum (1RM) for leg extension and leg curl exercises conducted at -28 d, -25 d, +72 h (n = 24), and +168 h (n = 22). PRO = milk protein supplement group, CON = isoenergetic control supplement group. Data are reported as the group mean and standard deviation. **Significantly different to -28 d ($p < 0.01$);* †*Significantly different to -25 d ($p < 0.05$);* $^{\tau}$*Significantly different to +72 h ($p < 0.05$);* §*Significantly different in females vs males ($p < 0.01$).*

4.4.4. Muscle Soreness

There were no significant differences between groups in muscle soreness VRS score or the change from baseline in VRS score at any time-point (*Table S4.1*). Significant peak increases in VRS score were observed for females (1.7 ± 1.0; $p = 0.001$) and males (2.2 ± 1.7; $p = 0.011$), and while females reported greater muscle soreness at pre and post compared with males (*Figure 4.4.a*), the pre-peak change was not different between sexes ($p = 0.569$). Effect sizes revealed that females had smaller increases in soreness at +24–168 h compared with males (*Figure 4.6*).

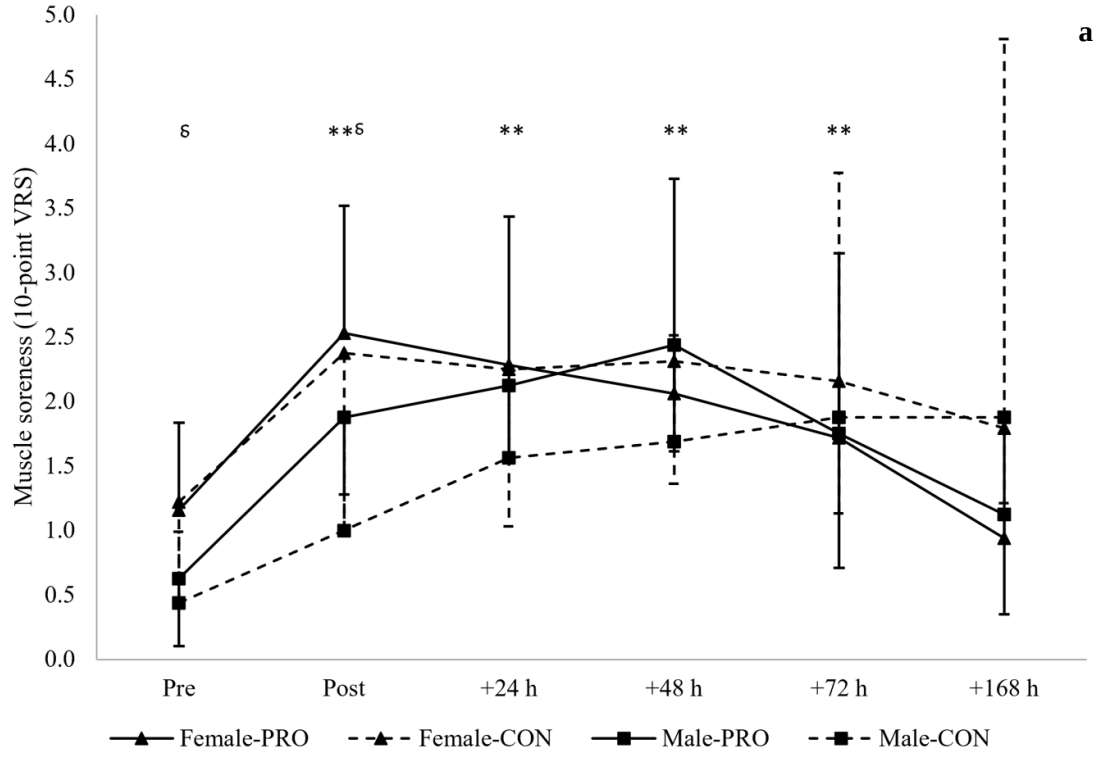
There were no significant differences between groups in PPT at any time-point and the change from pre-exercise was not different between MILK-PRO and CON at any time. Significant reductions in PPT were observed at +24 h ($p = 0.003$) and +48 h ($p = 0.006$) compared to pre (*Figure 4.4.b*) and the change at +48 h was greater in Male-CON compared with Female-CON and Female-PRO (*Table S4.1*). Significant peak reductions in PPT were found for both sexes, although the pre-peak change was significantly greater in males compared with females (-160.1 ± 104.3 vs. -51.0 ± 60.7 KPa; $p = 0.013$). Effect sizes indicated smaller decreases in PPT in females relative to males at all time-points (*Figure 4.6*).

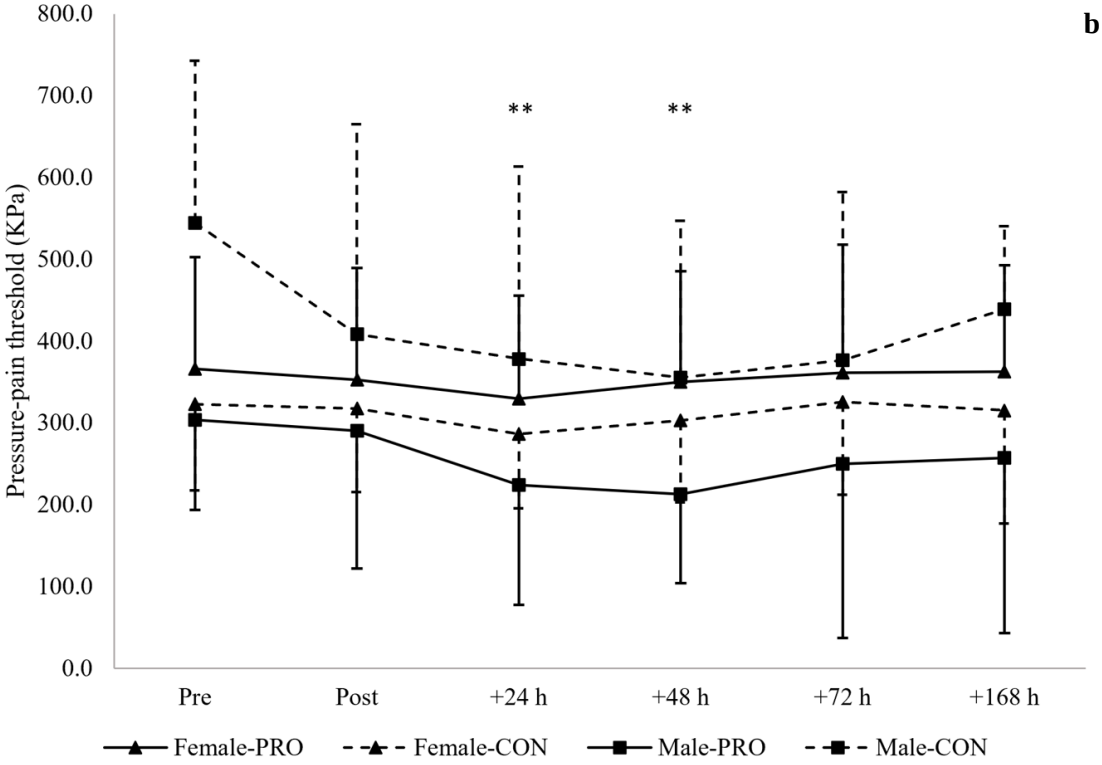
4.4.5. Leg Circumference

There were no significant differences between groups in leg circumference at any time-point. Leg circumference significantly increased over time (*Figure 4.4.c*) with a greater increase in Female-PRO compared with Female-CON at +24 h (0.8 ± 0.6 vs -0.3 ± 0.6; $p = 0.032$; *Table S4.1*) and in MILK-PRO compared with CON at +72 h (0.9 ± 1.0 vs 0.0 ± 0.6; $p = 0.020$). The pre-peak change in leg circumference was not different between groups or sexes (females vs. males: 1.1 ± 0.7 vs. 1.4 ± 0.9 cm). Based on ES, females experienced lesser increases in leg circumference than males at all time-points (*Figure 4.6*).

4.4.6. Range of Motion

There were no significant group*time interactions for ROM. Females had significantly greater ROM than males at most time-points (*Figure 4.4.d*). Peak reductions in ROM were not different between groups (*Table S4.1*) or sexes (-4.8 ± 4.3° and -3.8 ± 4.0° in females and males, respectively; $p = 0.610$). Effect sizes indicate that the decrease in ROM was greater in females at post and males at +24–168 h (*Figure 4.6*).


a
Muscle soreness (10-point VRS)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ε
**ε
**
**
**
Pre
Post
+24 h
+48 h
+72 h
+168 h
Female-PRO
Female-CON
Male-PRO
Male-CON


b
Pressure-pain threshold (KPa)
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
**
**
Pre
Post
+24 h
+48 h
+72 h
+168 h
Female-PRO
Female-CON
Male-PRO
Male-CON

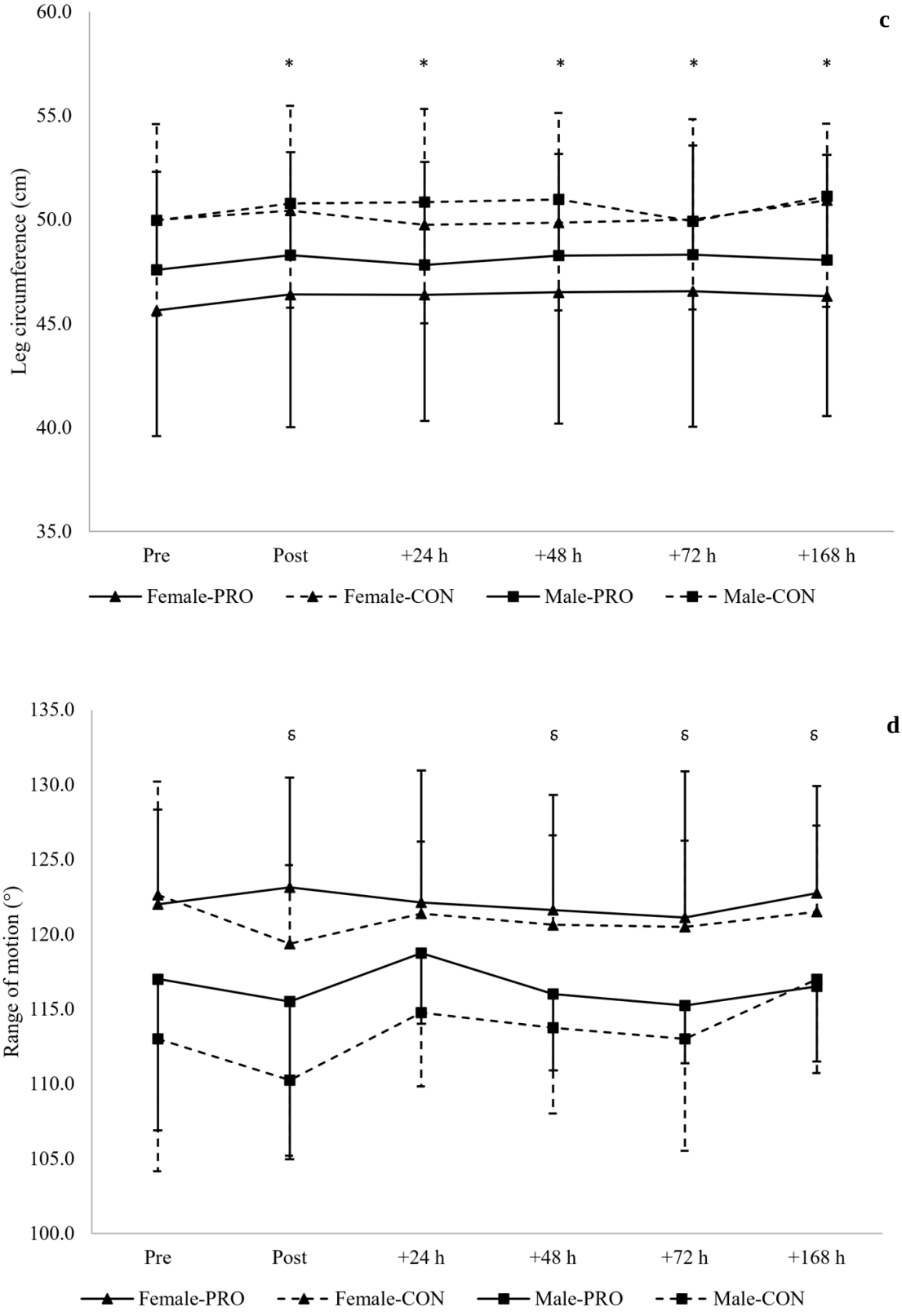


Figure 4.4. a. Muscle soreness visual rating scale score (mean values of quadricep, hamstring, glute, and calf muscles); b. pressure-pain threshold (mean of lower, mid, and upper leg sites); c. leg circumference (mean of lower, mid, and upper leg sites); d. leg range of motion (relaxed minus flexed knee joint angles). Measures conducted at pre, post, +24, +48, +72 h (n = 24), and +168 h (n = 22).

*PRO = milk protein supplement group, CON = isoenergetic control supplement group. Data are reported as the group mean and standard deviation. *Significantly different to pre ($p < 0.05$); **Significantly different to pre ($p < 0.001$); ᵟSignificantly different in females vs males ($p < 0.05$).*

4.4.7. Creatine Kinase

One outlying participant was identified (1287% increase from pre to +72 h). Removal of these data from the analysis did not impact significance levels and so presented data include this participant. Serum [CK] increased at +72 h relative to pre-exercise ($p = 0.011$) (*Figure 4.5*) with a greater increase in MILK-PRO than CON ($p = 0.031$). Compared with females, males had higher serum [CK] at post ($p = 0.012$) and a greater pre-post increase ($p = 0.018$). The pre-peak change in [CK] was greater in Male-PRO than Female-PRO ($p = 0.024$) and Female-CON ($p = 0.016$) (*Table S4.1*). With supplement groups combined, the pre-peak change in [CK] was significantly greater in males (940,086 ± 802,402 pg•mL^{-1}) compared with females (117,194 ± 220,152 pg•mL^{-1}) ($p = 0.031$). Based on ES, females experienced smaller [CK] elevations at post and +48–168 h (*Figure 4.6*).

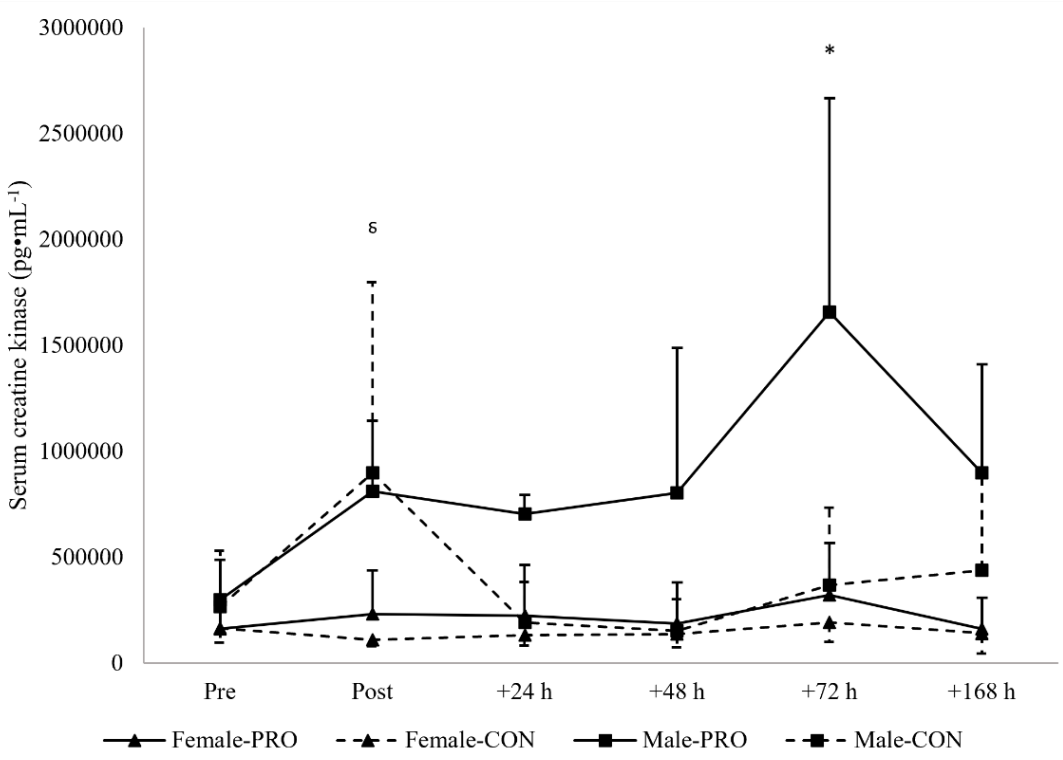


*Figure 4.5. Serum creatine kinase concentration analysed at pre (n = 20), post (n = 15), +24 h (n = 13), +48 h (n = 19), +72 h (n = 18), and +168 h (n = 14). PRO = milk protein supplement group, CON = isoenergetic control supplement group. Data are reported as the group mean and standard deviation. *Significantly different to pre ($p < 0.05$); ᵟSignificantly different in females vs males ($p < 0.05$).*

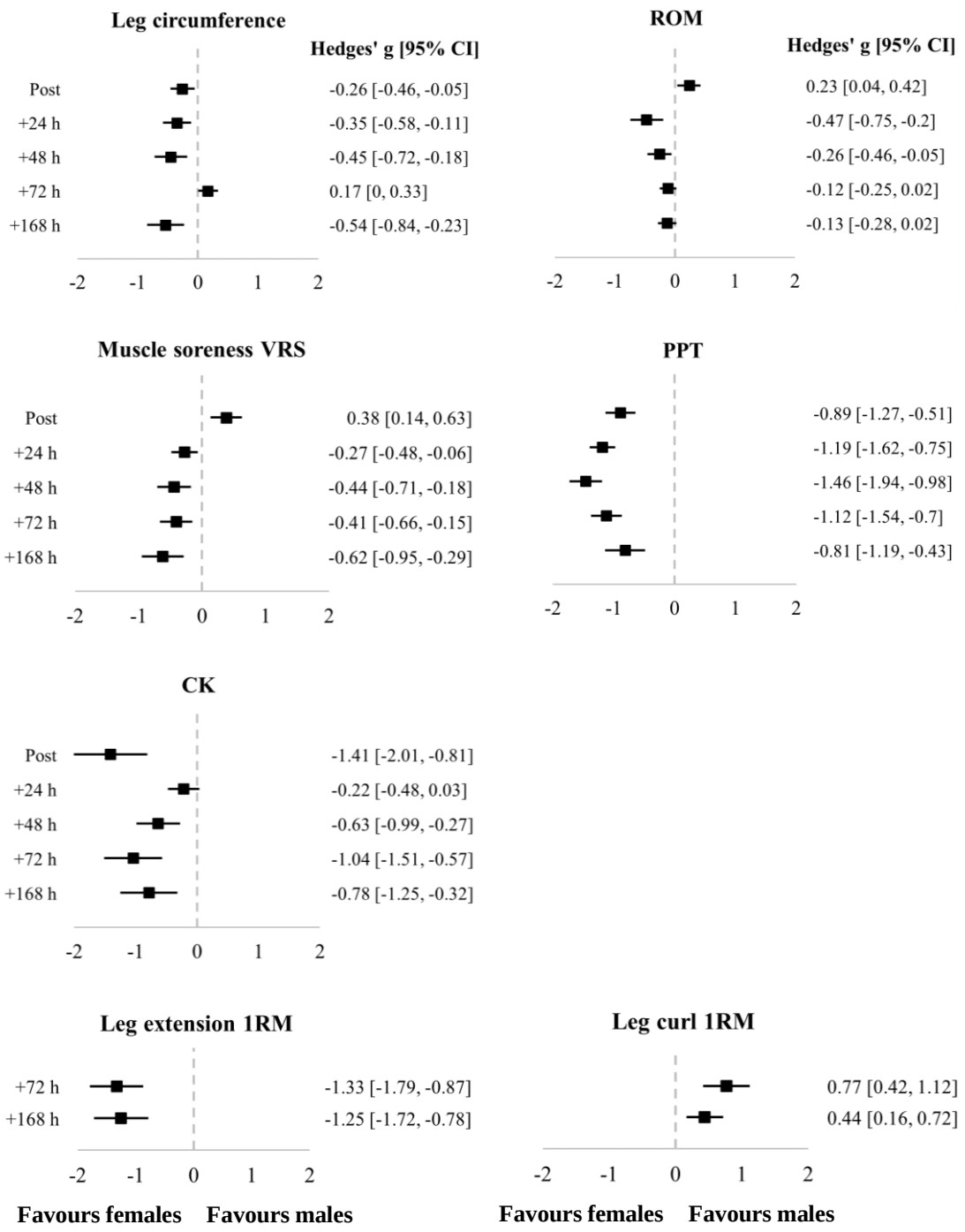


Figure 4.6. Hedges' g effect sizes with 95% confidence intervals for the mean change from pre-exercise to each post-exercise time-point between males and females for each muscle damage marker. ROM = range of motion, VRS = visual rating scale, PPT = pressure-pain threshold, CK = creatine kinase, 1RM = one-repetition maximum.

4.4.8. Correlations

Spearman's *Rho* correlation coefficients for the associations between EIMD markers and key body composition outcomes in females and males are displayed in *Table 4.5* and *Table 4.6*, respectively. In females, there were significant high negative associations between the peak change from pre-exercise in serum [CK] and body mass (r_s = -0.846, p = 0.001, n = 12), body mass index, LBM, BF%, and dominant and non-dominant leg lean mass and percent fat mass (*Figure 6.3*), and very high negative associations between serum [CK] and fat mass (r_s = -0.902, p < 0.001, n = 12), dominant and non-dominant leg fat mass (r_s = -0.916 and -0.935, respectively, p < 0.001, n = 12), and dominant and non-dominant leg mass (*Figure 4.7*). No significant associations existed for males.

Table 4.5. Associations between EIMD and body composition outcomes in females

		Total body			Dominant leg		
		BMI (kg•m^{-2})	Lean mass (kg)	Body fat (%)	Mass (kg)	Lean mass (kg)	Body fat (%)
1RM (kg)							
Leg extension	r_s	-0.221	-0.265	-0.108	0.063	0.124	-0.062
	p	0.411	0.320	0.691	0.816	0.646	0.819
Leg curl	r_s	-0.140	-0.175	-0.097	0.069	0.169	-0.078
	p	0.606	0.516	-0.720	0.798	0.531	0.775
PPT (KPa)	r_s	0.016	-0.009	-0.079	-0.227	-0.159	-0.271
	p	0.953	0.974	0.770	0.398	0.557	0.311
VRS	r_s	0.398	0.237	0.315	0.298	0.166	0.397
	p	0.127	0.377	0.235	0.262	0.538	0.128
Leg circ. (cm)	r_s	0.035	0.097	0.096	0.232	0.239	0.121
	p	0.898	0.720	0.724	0.387	0.373	0.655
ROM (°)	r_s	-0.230	-0.069	-0.281	0.038	0.106	-0.139
	p	0.391	0.798	0.292	0.887	0.695	0.608
CK (pg•mL^{-1})	r_s	-0.849	-0.804	-0.888	-0.942	-0.832	-0.867
	p	<0.001*	0.002*	<0.001*	<0.001*	0.001*	<0.001*

r_s = Spearman's Rho correlation coefficient, EIMD = exercise-induced muscle damage, BMI = body mass index, 1RM = one-repetition maximum, PPT = pressure-pain threshold, VRS = visual rating scale, circ. = circumference, ROM = range of motion, CK = creatine kinase, EIMD data represent the peak change from pre-exercise, n = 12 for CK, n = 16 for all other variables.

**Statistically significant at the level of p < 0.05*

Table 4.6. Associations between EIMD and body composition outcomes in males

		Total body			Dominant leg		
		BMI ($kg \cdot m^{-2}$)	Lean mass (kg)	Body fat (%)	Mass (kg)	Lean mass (kg)	Body fat (%)
1RM (kg)							
Leg extension	r_s	0.109	-0.546	0.109	-0.109	-0.218	0.109
	p	0.797	0.162	0.797	0.797	0.604	0.797
Leg curl	r_s	-0.359	-0.311	-0.431	-0.479	-0.240	-0.467
	p	0.382	0.453	0.286	0.230	0.568	0.243
PPT (KPa)	r_s	0.048	-0.167	0.286	0.024	-0.024	0.119
	p	0.911	0.693	0.493	0.955	0.955	0.779
VRS	r_s	0.073	0.073	-0.024	0.146	-0.293	-0.024
	p	0.863	0.863	0.954	0.729	0.482	0.954
Leg circ. (cm)	r_s	-0.049	-0.122	0.195	0.000	-0.049	0.146
	p	0.909	0.774	0.643	1.000	0.909	0.729
ROM (°)	r_s	-0.241	0.193	-0.241	-0.169	0.084	-0.169
	p	0.565	0.647	0.565	0.690	0.843	0.690
CK ($pg \cdot mL^{-1}$)	r_s	-0.095	-0.690	-0.190	-0.214	-0.190	-0.119
	p	0.823	0.058	0.651	0.610	0.651	0.779

r_s = Spearman's Rho correlation coefficient, EIMD = exercise-induced muscle damage, BMI = body mass index, 1RM = one-repetition maximum, PPT = pressure-pain threshold, VRS = visual rating scale, circ. = circumference, ROM = range of motion, CK = creatine kinase, EIMD data represent the peak change from pre-exercise, n = 8 for all variables.

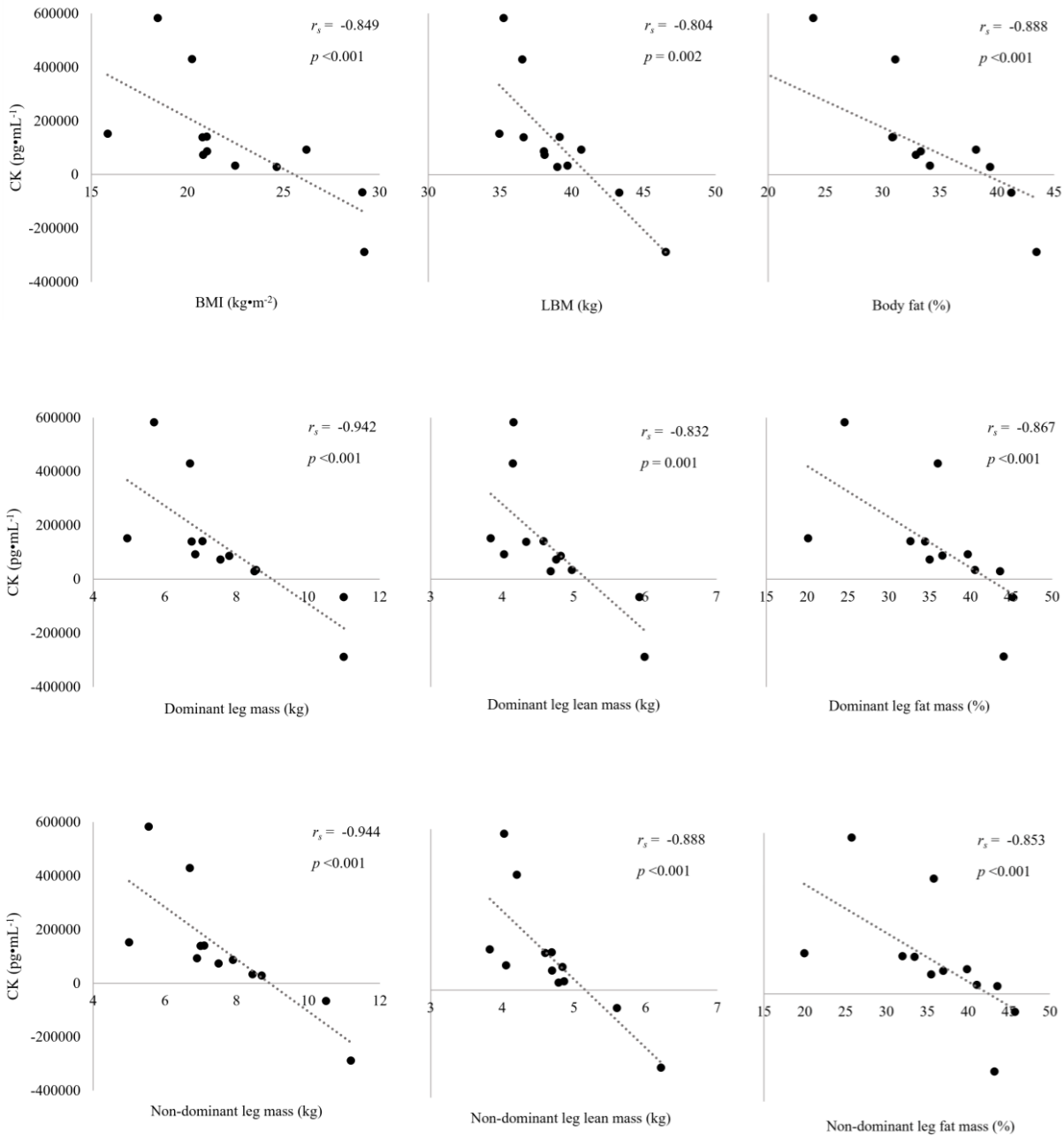


Figure 4.7. Associations between the peak change from pre-exercise in serum creatine kinase (CK) and body composition outcomes in females (n = 12).

r_s = Spearman's Rho correlation coefficient, BMI = body mass index, LBM = lean body mass.

4.5.0. Discussion

This study aimed to first, investigate whether milk protein consumption impacts resistance EIMD; second, elucidate whether EIMD responses differ between untrained males and females; and third, explore the relationship of EIMD and DXA-derived total and regional body composition. It was demonstrated that a single bout of habitual-type leg-based resistance exercise induced mild muscle damage without impairing maximal strength. In contrast with previous research (Cockburn et al., 2008; 2010; Draganidis et al., 2017; Rankin et al., 2015), no attenuative impact of milk protein ingestion on EIMD was documented. Exercise elevated [CK], reduced pain threshold, and prolonged muscle soreness more so in males than females, although changes in muscle swelling, flexibility, and strength were comparable between sexes. Further, it was indicated that exercise-induced peak increases in serum [CK] were negatively associated with several body composition outcomes in females, but not in males. In contrast to previous observations (Hickner et al., 2001; Margaritelis et al., 2019; Paschalis et al., 2013) no associations between body composition and the change in other EIMD markers, including muscle soreness, ROM, and maximal strength, were found in the current study. Overall, this study demonstrated that milk protein was not an effective nutritional strategy to mitigate mild resistance EIMD, and some symptoms of EIMD were attenuated in females relative to males; however, this does not appear to be attributed to sex differences in body composition.

Experimental models of EIMD have typically aimed to maximise muscle damage via extreme and unrealistic exercise protocols. Such protocols have included the performance of many (≥10) sets of one exercise (Burnley et al., 2010; Draganidis et al., 2017; Philpott et al., 2018; Wojcik et al., 2001), prolonged duration of continuous contractions (Nosaka et al., 2002_b), or exclusively eccentric contractions (Dale et al., 2015; Farup et al., 2014; Ives et al., 2017; Kim et al., 2017) which does not reflect habitual resistance training (ACSM, 2009). These exercise protocols have shown to induce severe muscle damage with ~50-fold increases in plasma [CK] (Nosaka & Newton, 2002) and sustained (7 days) strength decrements (Byrne & Eston, 2002; Farup et al., 2014). While these study designs allow for proof of concept and easier identification of EIMD management strategies, they lack ecological validity. Accordingly, the present study involved realistic exercise and demonstrated that a mildly stressful stimulus induced muscle damage, marked by elevated muscle soreness, swelling, CK activity, and reduced flexibility, without compromising muscle strength.

In contrast to the present study, significant strength declines following leg-based resistance exercise have been reported in untrained males (Buckley et al., 2010; Burnley et al., 2010; Cooke et al., 2010; Dale et al., 2015; Farup et al., 2014; Ives et al., 2017; Saracino et al., 2020; White et al., 2008; Wojcik et al., 2001) and females (Brown et al., 1997; Hicks et al., 2016; Paschalis et al., 2013), which is deemed the best indicator of EIMD (Damas et al., 2016$_a$; Paulsen et al., 2012; Warren et al., 1999). Nonetheless, peak strength loss occurs immediately post-exercise (Clarkson & Hubal, 2002; Clarkson et al., 1992) and here, strength assessments were not conducted until 72 and 168 h post-exercise to examine the impact of muscle damage on maximal strength at the time the muscle group would generally be re-exercised (i.e. after ≥2 d rest); thus, any strength deficits may have been restored. Therefore, maximal strength changes did not best indicate the magnitude of EIMD in the present study.

Leg extension and curl 1RM increased between baseline assessments, which is expected for those naïve to resistance exercise (McCurdy et al., 2004; Ritti-Dias et al., 2011). Initial strength gains in response to new exercise stimuli involve a learning effect, such as improvements in form and posture during movement. Hence in the present study, the participants' familiarity and confidence in performing the exercise may have driven strength improvements from -28 to -25 d. In addition, exercise adaptation occurs at a neural level to promote early-stage strength gains (Gabriel et al., 2006). Although the presence of neurological adaptation cannot be confirmed in the current study, evidence of increased motor unit firing rate between the first and second bout of leg extension exercise, concurrent with a 16% improvement in maximal force production, has been provided in untrained young adults (Kamen & Knight, 2004). Maximal strength increased further after the damaging exercise bout compared with baseline in MILK-PRO; however, this change was within the measurement error for the 1RM test (Grgic et al., 2020) and cannot be deemed a true increase. This suggests that first, one familiarisation session was sufficient for participants to achieve a reliable 1RM and second, that muscle damage was not so severe that maximal strength decrements occurred. Therefore, the findings of this study indicated that, in untrained males and females, mild muscle damage did not hinder the early-stage neurological adaptation required to prepare the muscle for future morphological adaptation.

Ingested milk protein did not attenuate EIMD at the dose provided in the present study. Exercise increased muscle soreness VRS ~2-fold both in MILK-PRO and CON; hence, conflicting Rankin et al. (2015), in which trained males and females benefited from ingested milk protein (17 g) for reducing passive and active muscle soreness 72 h following leg-based

resistance exercise. The small increase in muscle soreness rating in the current study may have limited the ability to detect meaningful between-group differences. Nonetheless, other studies reported comparable muscle soreness between protein and control groups despite ~5-fold (Gee et al., 2019) and ~10-fold (Cockburn et al., 2008) increases. Therefore, the present outcomes might contrast Rankin et al. (2015) due to participant training status, as the current study presents novel findings on untrained individuals. Equivocal data have been produced from previous studies conducted with trained males. No effect of milk protein relative to carbohydrate or water ingestion for reducing post-exercise muscle soreness was found by Cockburn and colleagues (Cockburn et al., 2008; 2012; 2013), whereas soreness was lowered by a milk-based protein-carbohydrate beverage consumed pre- or post-exercise (Cockburn et al., 2010). A single peri-exercise dose of milk protein was included in these studies, unlike the present study, which provided multiple supplement doses over several days. Comparably, milk protein concentrate was provided for 8 days post-exercise by Draganidis et al. (2017), which more rapidly alleviated muscle soreness relative to carbohydrate. However, milk protein did not attenuate soreness until 4–5 days post-exercise (Draganidis et al., 2017), and it is therefore plausible that the present study may have observed differences in soreness between MILK-PRO and CON had measures been taken at these time-points.

One possible explanation for why in this study, unlike others (Cockburn et al., 2010; Draganidis et al., 2017; Rankin et al., 2015), milk protein supplementation did not attenuate muscle soreness relates to participant blinding. In these previous studies, participants were aware of their treatment condition and so bias may have arisen within subjective measures if participants believed the ingested protein should alleviate their muscle soreness. By providing a low-protein yoghurt, similar in taste and appearance to the high-protein yoghurt, as opposed to water or a carbohydrate control beverage as used previously (Cockburn et al., 2010; Draganidis et al., 2017; Rankin et al., 2015), this limitation was overcome in the present study. By this means, the current findings may more accurately represent the impact of milk protein consumption on muscle soreness.

An unexpected finding was that significantly greater [CK] elevations 72 h post-exercise were experienced by males consuming milk protein compared with males consuming carbohydrate, which contrasts previous observations (Rankin et al., 2015). While difficult to explain, one explanation is that resting and post-exercise CK is highly variable and some individuals, termed high responders, experience significantly greater exercise-induced increases in CK activity compared to others, i.e. low responders (Clarkson & Ebbeling, 1988; Damas et al., 2016$_a$; Kim

& Lee, 2015; Nosaka & Clarkson, 1996$_c$). Here, [CK] increased from pre-exercise by >250% at +72 h in all Male-PRO participants and only one Male-CON participant. However, these participants were not high-responders to other EIMD outcomes and did not possess distinguishable characteristics from other participants. Thus, it seems the heightened [CK] peak at +72 h in Male-PRO is simply due to inter-individual variability and highlights the limitation of using [CK] to judge EIMD severity.

Despite disagreement (Dannecker et al., 2012; Hubal & Clarkson, 2009; Morawetz et al., 2020; Sayers & Clarkson, 2001), EIMD can be attenuated in females (Fernandez-Gonzalo et al., 2014; Kerksick et al., 2008; Minahan et al., 2015; Sewright et al., 2008), which has been attributed to protective effects of oestrogen on myofibre integrity (Tiidus, 2003). In the present study, measurements in females were conducted during the late-follicular phase of the MC when plasma oestrogen concentration is elevated, albeit not peaked (Owen, 1975). Oestrogen has shown to maintain myofibre membrane permeability following tissue injury (Bär et al., 1988; Tiidus, 2003) and hence might explain why peak elevations in post-exercise [CK] were attenuated in females compared with males, akin to previous observations (Fernandez-Gonzalo et al., 2014; Hicks et al., 2016; Minahan et al., 2015). However, this association is speculative here, as neither oestrogen concentration nor ultrastructural myofibre damage were measured.

Sex differences were also identified for subjective symptoms of EIMD. Pain threshold was unaffected by exercise in females, yet significantly reduced until +72 h in males. Exercise-induced muscle soreness is potentially driven by metabolic events and disruption of the extracellular matrix (Stauber et al., 1990). Following an exercise-induced increase in myofibre membrane permeability, the calcium-mediated mast cell degranulation and subsequent histamine release sensitises nociceptors and increases pain sensations (Marchettini et al., 1996; Stauber et al., 1990). These mechanisms were supported by the present study as relative to females, males experienced elevated CK leakage – indicative of weakened membrane integrity – concurrent with lower pain threshold. Meanwhile, mechanically-driven changes in muscle strength and flexibility were indifferent between sexes. Therefore, the current findings indicate a female protection against EIMD, perhaps specific to metabolically-induced muscle damage and mediated by oestrogen.

Compared with females, males possessed greater maximal strength and consequently, during the muscle damaging exercise bout utilised a higher absolute exercise load and performed a greater work volume. Performing a higher volume of elbow flexor exercise has been associated

with larger increases in muscle soreness, swelling, [CK], and decrements in strength and ROM than a lower volume of load-equated exercise in males (Howatson et al., 2007; Nosaka et al., 2001_b). However, when exercise load is not matched between comparator groups – as was the case here between males and females – higher compared with lower exercise volumes have not always been linked to more severe muscle damage (Arazi & Asadi, 2018; Draganidis et al., 2013). Therefore, the sex differences in pain threshold and [CK] identified in the present study could be mediated by dissimilarities in exercise volume, oestrogen status, or other undetermined factors, though nonetheless highlight the need for further sex-comparative and female-focussed research.

This study explored the relationship of body composition and EIMD in attempt to ascertain the probable causes of sex differences in muscle damage responses. Previous investigations of body composition in the context of EIMD have postulated that overweight and obese individuals are perhaps more vulnerable to EIMD owing to chronic inflammation and lipid accumulation within skeletal muscle, leading to structural alterations and disruption to myofibres. By this means, greater strength decrements, muscle soreness, and [CK] elevations, alongside reduced flexibility following damaging resistance exercise have been suffered by overweight/obese males and females relative to healthy weight individuals (Kim & So, 2018; Paschalis et al., 2010; 2013; Salvadori et al., 1993). In the present study, a higher BMI was associated with an attenuated post-exercise CK response in untrained, non-obese females. Opposingly, it was reported by Kim and So (2018) that untrained males with high BMI (≥25 $kg•m^{-2}$) had significantly elevated serum [CK] 24–96 h after eccentric elbow flexor exercise compared to males with lower BMI (18.5–22.9 $kg•m^{-2}$). Whereas, up to 24 h after whole-body resistance exercise, plasma [CK] was comparable among lean and obese sedentary males (Comstock et al., 2013). However, peak [CK], which is typically measured 3–5 days post-exercise (Clarkson & Hubal, 2002), was likely not captured here. These discrepancies may indicate sex differences or perhaps suggest that body composition is a more important factor in the relationship to EIMD than is BMI.

The supposed negative impact of overweight/obesity on EIMD has been attributed to the additional metabolic and mechanical strain placed upon muscle, connective, and bone tissues by adipose tissue (Hickner et al., 2001). Excess adiposity increases membrane phospholipid packing density, thereby weakening cell membrane structure (Andersson et al., 2002; Hazel & Williams, 1990; Knoblauch et al., 2013) and increasing the release of intramuscular proteins into systemic circulation. Accordingly, and in contrast to the current study, there have been

several reports of heightened [CK] following exercise in males and females with higher compared to lower body fat (Heled et al., 2007; Kim & Lee, 2015; Paschalis et al., 2010; 2013; Yoon & Kim, 2020). For instance, blood [CK] was 13-fold greater in males with ~25% compared with ~12% BF succeeding eccentric elbow flexions (Yoon & Kim, 2020). Following similar exercise, the greatest peak [CK] elevations were measured in males with ~16% BF and the lowest [CK] in males with ~13% BF by Kim and Lee (2015). However, these males would not be considered to possess excess adiposity, and therefore, these findings emphasised that high exercise-induced CK responses occur in individuals with healthy body fatness. In support, peak increases in serum [CK] of >1300% were documented by Paschalis et al. (2013) following eccentric leg extension exercise, which were comparable among untrained females categorised as underweight (~15% BF), lean (~20% BF), or overweight (~31% BF). Whereas it was suggested by Margaritelis et al. (2019) that both females with low (10–20 %) and high (>30%) BF are susceptible to increased post-exercise [CK] relative to those with normal BF%. In the current study, post-exercise peak serum [CK] was negatively related to absolute and relative BF, despite most females having >30% BF. Noteworthy, disparate study outcomes may owe to different body composition assessment methods, which vary in their validity and reliability (for instance, BF% values derived from DXA are ~5% higher than values derived from BIA) (Schubert et al., 2019). Overall, these findings indicated that, in non-obese females, higher body fatness is not detrimental to cell membrane integrity following EIMD.

Creatine kinase efflux from skeletal muscle is a strong determinant of serum [CK] (Baltusnikas et al., 2015), and therefore, it may be questioned whether greater muscle mass exacerbates post-exercise [CK]. Contradicting this hypothesis, a negative relationship between total-body muscle mass and the peak change in post-exercise serum [CK] was found in females in the present study. This relationship has been examined in few other studies, although post-exercise peak [CK] elevations were not related with the BIA-derived muscle mass of untrained males by Kim and Lee (2015). Likewise, low compared to normal FFM and fat mass-to-FFM ratio in recreational team sport males was not associated with greater changes to plasma [CK] or other indices of muscle damage following plyometric exercise by Fernandes et al. (2020). The present study was the first to relate EIMD with DXA-derived regional body composition and identified that both dominant and non-dominant leg muscle mass were negatively correlated with peak increases in [CK]. One possible explanation is that those females with higher muscle mass may habitually perform more physical activity – despite being naïve to resistance exercise – compared to those with lower muscle mass, and exercise training can attenuate CK responses

(Vincent & Vincent, 1997). Nonetheless, it was suggested by Hicks et al. (2016) that factors aside from muscle mass influence the post-exercise CK response. Attenuated elevations in serum [CK] after eccentric leg extensions were experienced by females relative to males in their study, and this difference remained once [CK] was normalised to vastus lateralis cross-sectional area. Thus, these findings indicated that muscle mass of the exercised region alone did not determine post-exercise [CK] increases nor explain sex differences in this response.

Associations between body composition and EIMD have been observed in males (Hickner et al., 2001; Kim & Lee, 2015; Kim & So, 2018; Yoon & Kim, 2020) and females (Margaritelis et al., 2019; Paschalis et al., 2013) yet to date, no study has explored sex differences in this relationship, despite variations both in body composition and EIMD between sexes (Fredsted et al., 2008; Hicks et al., 2016; Kerksick et al., 2008; Minahan et al., 2015; Sewright et al., 2008; Staron et al., 2000). The relationship of CK activity and body composition was significant for females but not for males in the current study. This disparity might stem from differences in these variables between sexes (higher peak post-exercise [CK] change, greater LBM, and lower BF% in males compared with females), though more likely reflects the small sample size acquired for males, which increases the risk of obtaining a type II statistical error and is a limitation of this study (Button et al., 2013; De Winter et al., 2016). Noteworthy, the modest association between LBM and [CK] in males (r_s = -0.690) bordered on statistical significance (p = 0.058), and thus, had equal sample sizes been obtained for males and females and statistical power had been sufficient, sex differences may have dissipated. Nonetheless, the associations between [CK] and other body composition outcomes were low and non-significant in males, suggesting sex specificity in this relationship.

4.5.1. Limitations

The following study limitations should be considered when interpreting and implementing its outcomes. First, only indirect EIMD markers were used, which are variable, subjective, and cannot objectively quantify myofibre damage, unlike direct assessments, such as muscle biopsy sampling. Nevertheless, biopsy procedures themself can cause tissue damage (Malm et al., 2000). Second, serum oestrogen was not measured, and therefore, MC phase cannot be confirmed nor can it be inferred whether oestrogen impacted EIMD and sex differences. Further, HCs were used by four females, which can impact EIMD (Carter et al., 2001; Minahan et al., 2015), although these females were, by chance, evenly distributed between supplement groups and so outcomes should be unaffected. Third, dietary intake was not fully controlled,

although dietary intake data indicated no unexpected changes from baseline. However, dietary intake was not assessed at +96–144 h and any notable changes in dietary intake during this time relative to +0–72 h could have impacted the +168 h assessments. Fourth, the ELISA test used to determine [CK] had low intra-assay precision (CV = 20.7%) – likely due to pipetting and washing techniques – thus the low accuracy of [CK] data potentially influenced the relationship of [CK] and body composition outcomes. Finally, Male-PRO and Male-CON data were underpowered to detect significant between-group differences, as n = 5 per group were required. These data were pooled for analyses to detect differences between sexes and supplement groups.

4.5.2. Conclusion

This study aimed to determine the impact of milk protein ingestion on indirect markers of muscle damage following resistance exercise and explore whether this response differs between untrained males and females. To this end, it was demonstrated that peri-exercise milk protein ingestion was not beneficial for recovery from muscle damage induced by an ecologically-valid resistance exercise bout. Nonetheless, milk protein may still be consumed to enhance other elements of post-exercise recovery and future studies should investigate alternative management strategies for mild EIMD. It was revealed by the current findings that, despite mild muscle damage, post-exercise maximal strength can be preserved, allowing the continuation of high-quality exercise. As such, experimental models of EIMD should include exercise protocols that mimic habitual training to increase the application of study outcomes to real-world settings. Furthermore, attenuated muscle damage responses to unaccustomed exercise were experienced by females relative to males in this study, highlighting the need for further research conducted with both sexes.

To explore the probable contributors to these sex differences, this study also aimed to elucidate the relationship of EIMD and total and regional body composition. Negative associations between the peak increase in post-exercise serum [CK] and total- and regional- body composition were found in untrained females, but not males. In contrast to previous observations, increased adiposity in non-obese females was demonstrated to have a positive rather than negative impact on myofibre membrane integrity following muscle damage. Further, due to the negative association between [CK] and LBM, it was refuted that the observed sex differences in EIMD were attributed to greater muscle mass in males compared with females. Further studies examining the relationship of body composition and EIMD in

males and females are warranted to confirm sex differences, as the present study was limited by its small sample size.

Overall, this study has contributed to current knowledge by showcasing that milk protein is ineffective for attenuating mild EIMD, and to this end, alternative management strategies for mild EIMD should be investigated. Furthermore, this study indicated that body composition is not a contributory factor to sex differences in EIMD, and thus, other influential factors ought to be explored in future research.

4.6.0. Supplementary Materials

Table S4.1. Mean change from pre-exercise to each post-exercise time-point for indirect markers of muscle damage

	Female-PRO (n = 8)	Female-CON (n = 8)	Male-PRO (n = 4)	Male-CON (n = 4)	Overall time effect
1RM (kg)					
Leg extension					
Pre-72 h	2.0 ± 5.1	0.6 ± 4.6	2.3 ± 4.7	-11.4 ± 22.9	$p = 0.241$
Pre-168 h	2.8 ± 4.0	3.2 ± 6.7	2.3 ± 4.7	-7.9 ± 15.9	$p = 0.152$
Pre-Peak	3.1 ± 3.8	3.0 ± 5.7	2.3 ± 4.7	-7.9 ± 15.9	$p = 0.068$
Leg curl					
Pre-72 h	1.3 ± 2.2	2.2 ± 3.1	6.2 ± 5.9	2.6 ± 12.1	$p = 0.023^{*}$
Pre-168 h	2.1 ± 3.3	3.5 ± 4.9	4.4 ± 3.1	5.3 ± 13.8	$p = 0.010^{*}$
Pre-Peak	2.7 ± 2.1	3.4 ± 4.4	7.4 ± 5.3	5.8 ± 13.8	$p = 0.004$*
PPT (KPa)					
Pre-Post	-3.9 ± 44.2[Ꝟ]	-5.1 ± 64.1[Ꝟ]	-13.0 ± 100.0	-136.5 ± 106.6	$p = 0.118$
Pre-24 h	-29.4 ± 65.5[Ꝟ]	-36.1 ± 58.5	-79.7 ± 93.1	-166.5 ± 104.4	$p = 0.002^{*}$
Pre-48 h	-12.3 ± 58.6[Ꝟ]	-19.9 ± 90.2[Ꝟ]	-90.6 ± 56.1	-189.0 ± 126.4	$p = 0.011^{*}$
Pre-72 h	7.5 ± 81.4	2.5 ± 89.7	-53.8 ± 132.6	-168.1 ± 133.9	$p = 0.167$
Pre-168 h	16.9 ± 94.8	-6.2 ± 77.6	-46.6 ± 120.6	-105.5 ± 147.3	$p = 0.326$
Pre-Peak	-51.5 ± 56.5	-50.5 ± 68.5	-113.4 ± 68.6	-206.8 ± 121.9	$p < 0.001^{*}$
VRS					
Pre-Post	1.4 ± 0.9	1.2 ± 0.7	1.3 ± 1.0	0.6 ± 1.4	$p < 0.001^{*}$
Pre-24 h	1.1 ± 1.1	1.0 ± 0.7	1.5 ± 1.0	1.1 ± 0.6	$p < 0.001^{*}$
Pre-48 h	0.9 ± 1.8	1.1 ± 0.7	1.8 ± 1.2	1.3 ± 0.7	$p < 0.001^{*}$
Pre-72 h	0.6 ± 1.5	0.9 ± 0.8	1.1 ± 1.3	1.4 ± 1.9	$p = 0.002^{*}$
Pre-168 h	-0.2 ± 1.0	0.5 ± 0.5	0.5 ± 0.5	1.4 ± 3.1	$p = 0.176$
Pre-Peak	1.7 ± 1.3	1.6 ± 0.7	1.8 ± 1.2	2.6 ± 2.3	$p < 0.001^{*}$
Leg circ. (cm)					
Pre-Post	0.8 ± 0.4	0.4 ± 0.6	0.7 ± 0.7	0.8 ± 0.3	$p = 0.001^{*}$
Pre-24 h	0.8 ± 0.6	-0.3 ± 0.6[Ɣ]	0.2 ± 1.0	0.9 ± 0.5	$p = 0.033^{*}$
Pre-48 h	0.9 ± 1.0	-0.5 ± 0.7	0.7 ± 1.3	1.0 ± 0.9	$p = 0.027^{*}$
Pre-72 h	1.0 ± 0.9	-0.1 ± 0.8	0.8 ± 1.5	0.0 ± 0.4	$p = 0.027^{*}$
Pre-168 h	0.6 ± 0.4	-0.1 ± 1.2	0.5 ± 1.1	1.2 ± 1.3	$p = 0.037^{*}$
Pre-Peak	1.4 ± 0.9	0.9 ± 0.6	1.1 ± 1.0	1.6 ± 0.8	$p < 0.001^{*}$
ROM (º)					
Pre-Post	1.1 ± 3.0	-3.3 ± 5.6	-1.5 ± 3.1	-2.8 ± 4.9	$p = 0.138$

Pre-24 h	0.1 ± 3.7	-1.3 ± 5.6	1.8 ± 7.1	1.8 ± 4.3	$p = 0.837$
Pre-48 h	-0.4 ± 3.8	-2.0 ± 4.3	-1.0 ± 5.4	0.8 ± 3.6	$p = 0.325$
Pre-72 h	-0.9 ± 5.8	-2.1 ± 5.5	-1.8 ± 6.3	0.0 ± 3.7	$p = 0.239$
Pre-168 h	0.8 ± 4.0	1.5 ± 4.9	-0.5 ± 5.4	4.0 ± 7.7	$p = 0.238$
Pre-Peak	-4.6 ± 4.0	-4.9 ± 4.8	-4.5 ± 3.7	-3.0 ± 4.7	$p < 0.001^{*}$
CK (pg•mL^{-1})					
Pre-Post	n = 6 49,857 ± 239,727	n = 3 -97,482 ± 30,479	n = 3 442,157 ± 327,474	n = 3 452,283 ± 616,659	$p = 0.125$
Pre-24 h	n = 5 86,253 ± 183,532	n = 3 -75,016 ± 84,600	n = 2 281,780 ± 265,785	n = 3 -46,642 ± 436,956	$p = 0.600$
Pre-48 h	n = 6 65,697 ± 179,215	n = 5 -28,459 ± 50,884	n = 4 505,158 ± 496,493	n = 4 -45,249 ± 308,134	$p = 0.260$
Pre-72 h	n = 5 200,864 ± 257,598	n = 5 28,041 ± 95,234	n = 4 1,359,584 ± 840,455	n = 4 148,520 ± 447,002†	$p = 0.011^{*}$
Pre-168 h	n = 5 24,621 ± 220,892	n = 3 -47,240 ± 168,499	n = 3 527,480 ± 662,756	n = 3 145,809 ± 665,962	$p = 0.638$
Pre-Peak	n = 7 159, 802 ± 282,152†	n = 5 57,542 ± 79,023†	n = 4 1,359,584 ± 840,455	n = 4 520,587 ± 571,553	*p= 0.001**

1RM = one-repetition maximum, PPT = pressure-pain threshold, VRS = visual rating scale, circ. = circumference, ROM = range of motion, CK = creatine kinase. The baseline value for 1RM was determined as the highest value from the -28 and -25 d time-points. n = 6 for Female-CON at 168 h. Data are expressed as the group mean ± standard deviation. $^{\gamma}$Significantly different to Female-PRO ($p < 0.05$); †Significantly different to Male-PRO ($p < 0.05$); $^{\tau}$Significantly different to Male-CON ($p < 0.05$); *Statistically significant at the level of $p < 0.05$.

4.7.0. Link to Next Chapters

The RCT found that milk protein supplementation was not an effective management strategy for mild EIMD, and hence, alternative strategies require investigation. While it would have been advantageous at this stage to conduct another primary RCT exploring an alternative nutritional strategy for the management of EIMD, this was not possible in light of the Covid-19 pandemic and the restrictions this imposed, particularly pertaining to face-to-face human-centred laboratory research. Consequently, the decision to examine secondary data was made. Considering this thesis and several previous works had reviewed various nutritional domains in the context of EIMD, focus was shifted away from nutritional elements of exercise recovery and toward components of the exercise itself. Of the principle extrinsic exercise variables (i.e. load, set number, repetition number, inter-set rest period, and contraction type and velocity),

the choice was made to examine the impact of exercise load on EIMD with the aim of finding a mitigating strategy that would be suitable for novice exercisers to implement with ease.

5.1.0. Prelude

This thesis first considered nutritional elements in relation to EIMD and the focus then switched to the muscle-damaging exercise bout itself. Manipulating exercise load is a principal component of progressive resistance training. It is commonly believed that exercising with heavier weights induces greater muscle damage; however, there is currently no consensus evidence to support this notion. If utilising lighter weights induces less muscle damage while promoting comparable chronic training adaptations, this may be a viable, easy, and preferred option for many exercisers hesitant or unable to lift heavier weights. Therefore, scoping the existing literature surrounding resistance exercise load in the context of EIMD was considered important to aid the design of a novel study to address any identified knowledge gaps.

5.2.0. Introduction

Regular resistance exercise can maintain general health, enhance sport performance, offset age-associated sarcopenia, and modify body composition. Fundamentally, resistance exercise training may increase skeletal muscle mass, enhance strength, help maintain stability, improve glucose tolerance, and increase bone density (Anderson & Behm, 2005; Eriksson et al., 1998; Layne & Nelson, 1999; Schoenfeld, 2010; Tan, 1999). However, the muscle contractions associated with resistance exercise – particularly unaccustomed or eccentric contractions – induce damage to muscle fibres (Stauber, 1989). At a cellular level, damage to structural components of muscle sarcomeres and to the sarcolemma is evident and characterised by elevated concentrations of intramuscular proteins (e.g. CK and myoglobin) within systemic circulation (Allen, 2001; Duncan & Jackson, 1987; Morgan et al., 1996; Warren et al., 1993). On the surface, resistance exercise-induced muscle damage (EIMD) leads to muscle soreness, swelling, and impaired muscle function, e.g. reductions in muscle force generating capacity and flexibility, which can persist for up to 10 days following exercise (Clarkson & Hubal, 2002; Manfredi et al., 1991). Consequently, extended recovery periods between exercise bouts are required or exercise performance may suffer. Therefore, attenuating acute EIMD may promote frequent, quality exercise bouts to aid chronic muscle adaptations, such as skeletal muscle hypertrophy and strength gains (Damas et al., 2016$_b$).

Several EIMD management strategies have been proposed, which primarily target the pre- and post- exercise period, such as massage, stretching, cryotherapy, compression garments, electrostimulation (Dupuy et al., 2018; Torres et al., 2012), protein feeding (Pasiakos et al., 2014), and other nutritional supplements (Harty et al., 2019). Albeit less extensively reviewed, manipulating extrinsic exercise components, including the speed of muscle contraction (Chapman et al., 2008; Nogueira et al., 2013), ROM (Fochi et al., 2016; Nosaka et al., 2005; Váczi et al., 2009), inter-set rest period (Machado & Willardson, 2010; Mayhew et al., 2005), total work volume (repetitions × sets × load) (Chen et al., 2020; Hasenoehrl et al., 2017; Mendham et al., 2011; Peake et al., 2006), maximum force generated (Nosaka & Newton, 2002), and muscle group exercised (Chen et al., 2011) can also influence the magnitude of EIMD. Of particular interest is the relationship between muscle force and length during contraction. Characteristically, greater force outputs can be achieved at longer muscle lengths, until the muscle hyper-extends and force can no longer be maintained, i.e. the length-tension curve (Gordon et al., 1966). These variables have been considered in isolation to ascertain whether muscle force or length is determining the severity of EIMD. In accordance, studies

conducted with animals (Brooks et al., 1995; Talbot & Morgan, 1998) and humans (Fochi et al., 2016; Newham et al., 1988; Nosaka et al., 2005; Nosaka & Sakamoto, 2001; Váczi et al., 2009) have consistently reported greater EIMD when the targeted muscle is stretched over a wider range of muscle lengths. Therefore, utilising shorter muscle lengths during contraction helps reduce EIMD.

Maximum muscle force during contraction as a factor in EIMD is ambivalent. To this end, comparable reductions in maximum muscle strength (Barroso et al., 2011; Draganidis et al., 2013; Uchida et al., 2009) and elevations in muscle soreness (Arazi & Asadi, 2018; Hasenoehrl et al., 2017; Paschalis et al., 2005; Peake et al., 2006; Uchida et al., 2009) and intramuscular protein leakage (Arazi & Asadi, 2018; Bartolomei et al., 2017; Draganidis et al., 2013; Güzel et al., 2007; Hasenoehrl et al., 2017; Paschalis et al., 2005; Peake et al., 2006) following resistance exercise performed with varied loads by males have been reported in several studies. Nonetheless, many others have documented attenuated EIMD when resistance exercise is performed with lighter compared with heavier loads (Chen et al., 2020; Nosaka et al., 2002_b; Nosaka & Newton, 2002_a; Orssatto et al., 2018) and in this regard, reducing muscle force output by utilising lower exercise loads may be a viable strategy to mitigate EIMD.

Consistently utilising lower exercise loads has shown to induce comparable muscle adaptations to those obtained during traditional high-load resistance training (Cholewa et al., 2018; Dobson, 2021; Fink et al., 2018; Franco et al., 2019; Kubo et al., 2020; Lasevicius et al., 2019; Ribeiro et al., 2020; Schoenfeld et al., 2017_a; Stefanaki et al., 2019). Furthermore, performing resistance exercise with lower loads was perceptively less exerting compared to with higher loads (Arazi & Asadi, 2018; Richardson et al., 2018), which may increase exercise adherence and particularly suit individuals who are elderly, recovering from injury, or naïve to resistance exercise. Establishing whether the use of lower resistance exercise loads can ameliorate EIMD, while concomitantly promoting muscle adaptations, could provide new insights to inform training recommendations. Therefore, this scoping review aimed to determine whether performing low-load resistance exercise is an effective strategy for reducing EIMD by comparing indirect markers of EIMD (MVC, muscle soreness, and [CK]) in response to acute low-load and high-load resistance exercise performed by healthy adults.

5.3.0. Methods

5.3.1. Inclusion Criteria

The analysis was confined to studies published in English-language peer-reviewed journals that met the following criteria: (1) an experimental trial that included two or more load intensities of acute resistance exercise. Exercise intensity was classified as low-load (≤70% 1RM or ≥12RM) or high-load (>70% 1RM or <12RM). It was acknowledged that 70% 1RM may not typically be considered low intensity; however, preliminary reading of the EIMD literature revealed that several studies classified 70% 1RM as the lower load, compared to loads >70% 1RM (Barroso et al., 2011; Bartolomei et al., 2017; Draganidis et al., 2013). Therefore, to prevent exclusion of relevant studies from the review, the load boundaries were increased slightly; (2) included at least one indirect measure of muscle damage, i.e. blood biomarker(s), muscle soreness, muscle function (e.g. MVC, ROM); (3) muscle damage was measured ≥24 h following the exercise bout; (4) the study involved adult (≥18 y) participants of any age, sex, ethnicity, and training status with no known medical conditions or musculoskeletal injuries. Studies involving the use of vascular occlusion were excluded, as this may have confounded the impact of exercise load.

5.3.2. Search Strategy

The literature search was conducted in line with the PRISMA guidelines (Moher et al., 2009). Searches of PubMed and Google Scholar databases for studies published up to the date of November 2020 were conducted. The following syntax was used to conduct the search: ("resistance exercise" OR "resistance training") AND ("exercise load" OR "exercise intensity" OR "training load" OR "training intensity" OR "high load" OR "low load" OR "loading") AND ("muscle damage" OR "muscle soreness" OR "muscle function" OR "performance" OR "recovery" OR "creatine kinase"). The title, abstract, and full text of the retrieved articles were screened for eligibility by an independent reviewer. The reference list of relevant articles were also screened for eligible articles.

5.3.3. Coding of Studies

Studies were read and coded by a single reviewer for the following variables: (1) author, title, and year of publication: (2) participant demographics e.g. sex, age, training status, and sample size. Age was categorised as in the review by Schoenfeld et al. (2017_a); (3) resistance exercise protocol, including load intensity, mode of exercise, set and repetition number, and duration of

rest period; (4) methods used for the assessment of muscle damage; (5) time points of the measurements in relation to the exercise bout; (6) significant findings.

5.3.4. Methodological Quality

Quality assessment of each study was independently conducted based on the 11-point Physiotherapy Evidence Database (PEDro) scale, which has been considered a reliable and valid measure of the methodological quality of randomised controlled trials (Elkins et al., 2010; Maher et al., 2003). Given that blinding of investigators and participants is not possible during supervised resistance exercise trials, items 5–7 pertaining to blinding were removed from the scale, in accordance with Schoenfeld et al. (2017_a). Therefore, study quality was assessed by use of a modified 8-point PEDro scale and ratings were categorised as follows: 6–7 = "excellent"; 5 = "good"; 4 = "moderate"; and 0–3 = "poor".

5.4.0. Results

Of the articles initially reviewed, 21 were determined to potentially meet the inclusion criteria based on information contained within the abstracts. Following full-text review, 15 articles were confirmed to meet the inclusion criteria and were included in the review. *Figure 5.1* shows a flow chart of the literature search and *Table 5.1* summarises the studies included for analysis.

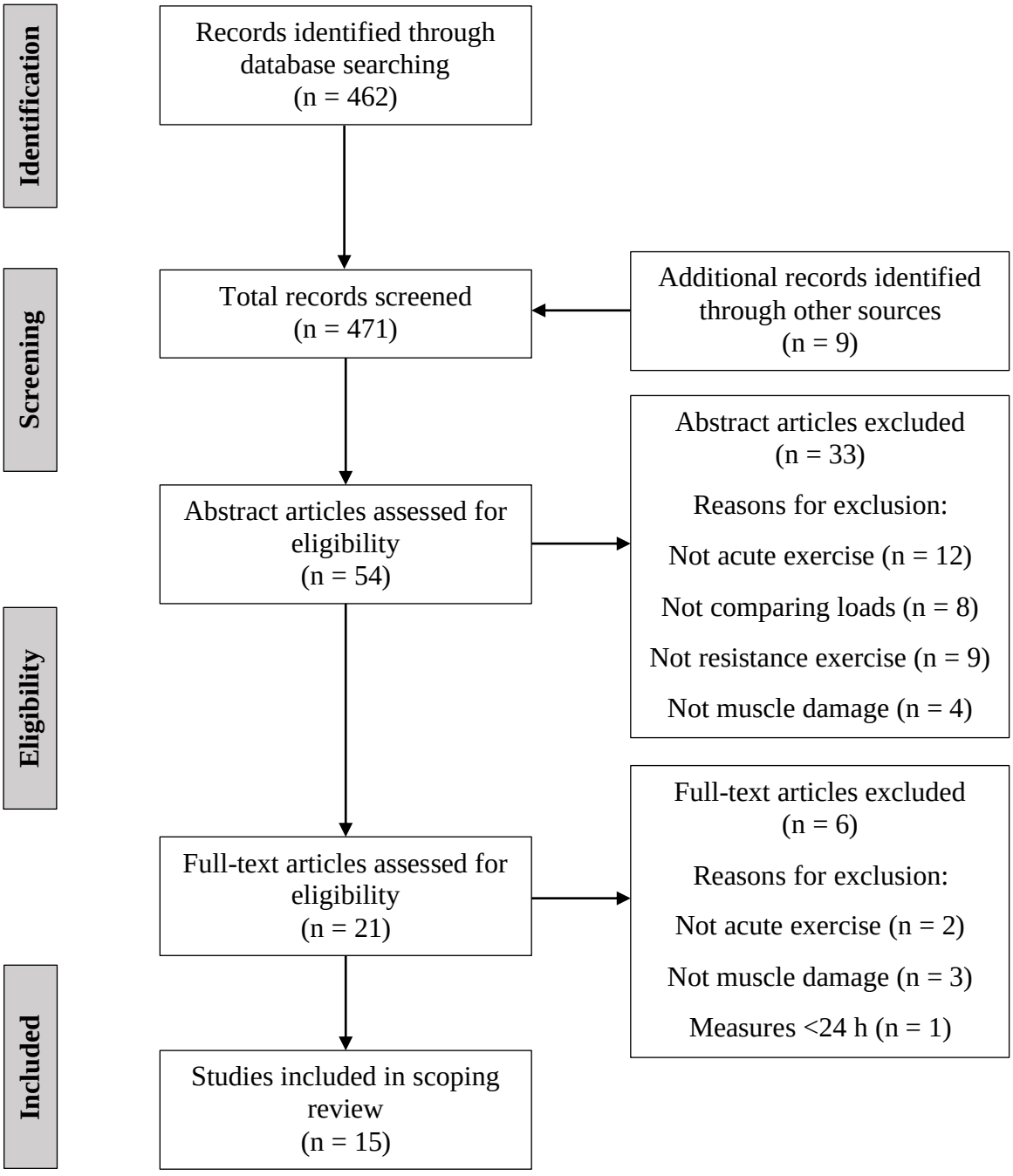


Figure 5.1. Flow diagram of literature search process.

Table 5.1. Summary of studies included in the scoping review (n = 15)

Reference	Study design Participant characteristics	Mode(s) of resistance exercise	Exercise protocol [load; sets × repetitions; ROM (inter-set rest period)]	Volume matched?	Markers of muscle damage	Time points of measures	Primary findings
Arazi and Asadi (2018)	Parallel n = 28 M; young; untrained	Leg press	LL: 60% 1RM; 4 × 21 (2 min) HL: 80% 1RM; 4 × 5 (2 min) HL: 100% 1RM; 4 × 1 (2 min) Non-exercising control	No	Plasma [CK and CRP] Muscle soreness VAS (during bodyweight squat)	Pre +24 h +48 h +72 h	↑[CK] at +24–72 h in HL 100% compared to HL 80% and LL ↑[CRP] at +24–72 h in HL 100% compared to LL ↑Muscle soreness at +24–72 h in HL 100% compared to HL 80% and LL
Barroso et al. (2011)	Parallel n = 24 M; young; untrained	Unilateral eccentric elbow flexor	LL: 70% 1RM; 5 × 6; 120° (2 min) LL: 70% 1RM; 10 × 6; 120° (2 min) HL: 110% 1RM; 5 × 6; 120° (2 min)	No	Muscle soreness VAS (rested) ROM elbow joint Upper arm CIRC 1RM	Pre Post +48 h +96 h	↓1RM at post in HL ↑Muscle soreness and ↓ROM at post and +48 h in HL

Bartolomei et al. (2017)	Crossover n = 12 M; young; trained	Back squat	LL: 70% 1RM; 8 × 10 (75 s) HL: 90% 1RM; 8 × 3 (3 min)	No	Serum [CK, Mb, LDH, IL-6, CRP] Muscle soreness VAS (rested) CMJ peak power Isometric MVC knee flexor Isokinetic MVC at 60° and 180° leg extension Isometric mid-thigh-pull peak force Isometric half squat peak force Vastus lateralis CSA and MT by ultrasound	Pre Post +30 min +24 h +48 h +72 h	↑[IL-6] at +30 min in LL ↑Muscle soreness at post to +72 h in LL ↓ Isometric MVC at +48–72 h; CMJ at +30 min and +48 h; and Isokinetic peak torque at 60° at +30 min and +24 h in LL ↑CSA at +30 min and +24–48 h in LL
Chen et al. (2020)	Parallel n = 36 M; young; untrained	Unilateral eccentric elbow flexor	LL: 10% MVC; >100 × 10; 90° (2 min) LL: 50% MVC; ≥4 × 5; 90° (2 min) HL: 100% MVC; ≥2 × 5; 90° (2 min)	No	Plasma [CK] Muscle soreness VAS (unloaded arm extension) Isometric MVC elbow flexor ROM elbow joint Upper arm CIRC	Pre Post +30 min +24 h +48 h +72 h +96 h +120 h	↑Peak [CK] and muscle soreness in HL vs LL ↓Isometric MVC at post was intensity-dependent (10% > 50% > 100%); as was recovery speed of isometric MVC and ROM

							from +24 to +120 h (10% > 50% > 100%)
Cornish et al. (2018)	Crossover n = 11 M; older adults; untrained	Chest press, shoulder press, seated row, leg press, leg extension, plantar flexion	LL: 60% 1RM; 2 × 12 (1 min) HL: 72% 1RM; 2 × 10 (1 min) HL: 80% 1RM; 3 × 6 (1 min)	Yes	Plasma [Mb and IL-6]	Pre Post +3 h +24 h +48 h	↔
Draganidis et al. (2013)	Crossover n = 10 M; young; trained	Back squat, leg extension, leg curl, lunge, calf raise	LL: 65–70% 1RM; 4 × 8–10 (1 min) HL: 85–90% 1RM; 4 × 4–6 (3 min) Non-exercising control	No	Serum [CK and CRP] Muscle soreness VRS (during palpation) ROM knee joint 1RM Football-specific skills (dribbling, passing, shooting, and heading)	Pre Post +30 min +24 h +48 h +72 h	↑[CRP] at post and +24 h in HL ↑Muscle soreness at post, +24 and +48 h in HL

Güzel et al. (2007)	Parallel n = 20 M; young; untrained	Back squat, leg extension, lat pull-down, chest press (circuit)	LL: 20–35% 1RM; 3 × 20–30 (1 min) HL: 80–95% 1RM; 3 × 2–8 (1 min)	No	Plasma [CK and NO_x]	Pre Post +6 h +24 h +48 h +72 h	↑[NO_x] at +6–48 h in HL
Hasenoehrl et al. (2017)	Crossover n = 15 M; young; trained	Eccentric elbow flexor	LL: 50% 1RM; 3 × to failure (30 s) HL: 100% 1RM; 3 × to failure (30 s)	No	Serum [CK, Mb and IL-6] Muscle soreness VAS (rested) Upper arm CIRC	Pre +24 h +48 h +72 h +96 h	↑Arm CIRC at pre to +96 h in HL
Mendham et al. (2011)	Crossover n = 12 M; middle-age; untrained	Chest press, shoulder press, lat pull-down, leg press, leg curl, leg extension, lunge	LL: 60% 1RM; 3 × 10 (90 s) HL: 80% 1RM; 3 × 10 (90 s)	No	Serum [CK, Mb, CRP and IL-6] Total leukocyte count	Pre Post +3 h +24 h	↑[IL-6] at post, [Mb] and total leukocyte count at +3 h, and [CK and CRP] at +24 h in HL

Nosaka and Newton (2002)	Crossover n = 8 M; young; untrained	Unilateral eccentric elbow flexor	LL: 50% isometric MVC; 3 × 10; 130° (3 min) HL: 100% isometric MVC; 3 × 10; 130° (3 min)	No	Plasma [CK] Muscle soreness VAS (during palpation and unloaded arm extension) ROM elbow joint Isometric MVC elbow flexor Upper arm CIRC Elbow flexor MT by ultrasound	Pre Post +24 h +48 h +72 h +96 h +120 h	↑[CK] at +48–120 h in HL ↑Peak soreness when muscle extended in HL ↓Isometric MVC at post to +120 h and ROM at +24–120 h in HL ↑CIRC and MT at +72–120 h in HL
Nosaka et al. (2002b)	Parallel n = 68 M; young; untrained	Unilateral elbow flexor	LL: 9% isometric MVC; 1 × ~3600; 120° (2 h continuous) HL: 100% isometric MVC; 12 × 1 eccentric; 90° (15 s)	No	Plasma [CK] Muscle soreness VAS (during palpation and unloaded arm extension and flexion) Isometric MVC elbow flexor Upper arm CIRC MT by ultrasound	Pre Post +1 h* +3 h* +6 h* +10 h* +24 h +48 h +72 h +96 h *LL only	↑[CK] and soreness in extended muscle at +24–96 h in HL ↓ Isometric MVC at post in LL and at +24–96 h in HL ↑Arm CIRC at post to +96 h in HL

Orssatto et al. (2018)	Parallel n = 22 (15 M, 7 F); older adults; untrained	Leg press, leg curl	LL: 60% 1RM; 3 × to failure (2 min) HL: 85% 1RM; 3 × to failure (2 min)	No	Isometric MVC knee extensor CMJ height 'Up and go' time Stair ascent and descent time	Pre Post +24 h +48 h +72 h	↓Isometric MVC at +24–48 h in HL
Paschalis et al. (2005)	Crossover n = 12 M; young; untrained	Unilateral eccentric knee extensor	LL: 50% eccentric MVC; continuous until volume-matched HL: 80% eccentric MVC; 12 × 10 (2 min)	Yes	Serum [CK] Muscle soreness VRS (during palpation) ROM knee joint Isometric MVC knee extensor Eccentric MVC	Pre +24 h +48 h +72 h +96 h	↑[CK] at +24 h in HL ↓ Isometric MVC at +24–96 h and EPT at +24 and +72–96 h in HL
Peake et al. (2006)	Crossover n = 10 M; young; untrained	Unilateral eccentric elbow flexor	LL: 10% isometric MVC; 10 × 60; 60° (1 min) HL: 100% isometric MVC; 10 × 3; 60° (3 min)	No	Plasma [CK, Mb and CRP] Serum [IL-6, IL-10 and TNF-α] Muscle soreness VAS (during palpation) and PPT test (with pressure algometry) Isometric MVC elbow flexor Upper arm CIRC	Pre Post +3 h +24 h +48 h +72 h +96 h	↓ Isometric MVC at +24–96 h and ROM at +48–96 h in HL

					ROM elbow joint		
Uchida et al. (2009)	Parallel n = 35 M; young; trained	Bench press	LL: 50% 1RM; 4 × 20 (2 min) HL: 75% 1RM; 5 × 11 (2 min) HL: 90% 1RM; 10 × 4 (2 min) HL: 110% 1RM; 8 × 4 eccentric-only (2 min) Non-exercising control	Yes	Plasma [CK, IL-6, IL-1β and TNF-α] Muscle soreness VAS (during palpation)	Pre +24 h +48 h +72 h	↔

Arrows indicate a significant difference between groups (↑ = higher, ↓ = lower, ↔ = no significant differences); participant age is classified as young (18–39 y), middle-aged (40–64 y), and older adults (≥ 65 y) (Schoenfeld et al., 2017$_a$); M = males; F = females; HL = high-load; LL = low-load; CK = creatine kinase; Mb = myoglobin; LDH = lactate dehydrogenase; IL = interleukin; TNF-α = tumor necrosis factor-alpha; CRP = C-reactive protein; NO_x = nitric oxide; 1RM = one repetition maximum; MVC = maximal voluntary contraction; VAS = visual analogue scale; VRS = visual rating scale; PPT = pressure-pain threshold; CMJ = counter movement jump; CSA = cross-sectional area; MT = muscle thickness; ROM = range of motion; CIRC = circumference.

5.4.1. Study Quality and Overview

The methodological quality rating of the included studies is presented in *Table 5.2.* The mean and median rating of study quality was five, indicating the pool of studies were of good quality. Only one study was categorised as poor quality and three as excellent. In total, 323 participants were included in the 15 studies, consisting of 21 trials. The majority of participants were male (98%) and in most studies (n = 8) were young and untrained. The resistance exercise protocol used to induce muscle damage included both eccentric and concentric muscle contractions in nine of the studies, and eccentric-only contractions in the remaining six studies. Most exercise protocols were restricted to upper-body muscle groups (n = 7 studies) with only three studies using whole-body resistance exercise (Cornish et al., 2018; Güzel et al., 2007; Mendham et al., 2011). In 13 studies, the total work volume of the exercise was prescribed, while in 2 studies, participants performed each exercise set to volitional failure (Hasenoehrl et al., 2017; Orssatto et al., 2018). Consequently, in only three studies was the total volume of the exercise matched between groups (Cornish et al., 2018; Paschalis et al., 2005; Uchida et al., 2009).

Table 5.2. Methodological quality rating of studies included in the scoping review (n = 15)

Reference	Total score (/7)	Rating
Arazi and Asadi (2018)	5	Good
Barroso et al. (2011)	4	Moderate
Bartolomei et al. (2017)	5	Good
Chen et al. (2020)	4	Moderate
Cornish et al. (2018)	6	Excellent
Draganidis et al. (2013)	5	Good
Güzel et al. (2007)	5	Good
Hasenoehrl et al. (2017)	5	Good
Mendham et al. (2011)	5	Good
Nosaka and Newton (2002)	5	Good
Nosaka et al. (2002_b)	4	Moderate
Orssatto et al. (2018)	6	Excellent
Paschalis et al. (2005)	4	Moderate
Peake et al. (2006)	3	Poor
Uchida et al. (2009)	7	Excellent

Among the studies reviewed, the most common measures used to assess muscle damage, which were obtained up to 120 h post-exercise, were subjective muscle soreness, MVC, and plasma or serum [CK]. Therefore, these muscle damage markers will be summarised and discussed.

5.4.2. The Impact of Exercise Load on Maximal Voluntary Contraction

Following EIMD, reductions in MVC are most debilitating to future exercise performance. Performing resistance exercise with lighter loads may reduce the post-exercise decline in maximal strength and accelerate its recovery. It was established by this review that shortly (<24 h) after resistance exercise performed with low loads, the decline in MVC was significantly attenuated (Barroso et al., 2011; Nosaka & Newton, 2002) or comparable (Draganidis et al., 2013; Orssatto et al., 2018; Peake et al., 2006) to when exercise was performed with high loads. While augmented strength loss <24 h following low- versus high- load resistance exercise was not reported in some studies (Bartolomei et al., 2017; Chen et al., 2020; Nosaka et al., 2002_b), the recovery of maximal strength toward baseline values occurred more rapidly during the days following low-load exercise (Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Orssatto et al., 2018; Peake et al., 2006). Thus, these outcomes suggested that resistance exercise performed with lower loads induced transient strength loss, while higher loads triggered more sustained strength decrements. Furthermore, low-load resistance exercise did not always cause significant strength loss from pre-exercise values (Barroso et al., 2011; Draganidis et al., 2013; Paschalis et al., 2005). Whereas, in only one instance was maximal strength preserved following high-load resistance exercise (Bartolomei et al., 2017). It likely follows that utilising lower loads during resistance exercise is beneficial for managing muscle strength loss associated with EIMD.

Lack of methodological control over other resistance exercise variables might explain why, in some cases (Bartolomei et al., 2017; Chen et al., 2020; Nosaka et al., 2002_b), exercise-induced strength loss was greater when lower loads were utilised. For example, augmented strength loss immediately following a series of elbow flexions performed with lower (9% of maximal isometric force [MIF]) compared to higher (100% MIF) loads was reported by Nosaka et al. (2002_b). However, there were dissimilarities between the two exercise protocols, additional to the exercise load. First, the low-load exercise included both concentric and eccentric muscle contractions, whereas the high-load exercise consisted only of eccentric muscle contractions. Second, due to different starting angles of the elbow joint, the total ROM of the muscle action was greater in the low-load compared to high-load condition (120 vs 90 °), and it is widely

accepted that joint ROM during muscle contraction influences the magnitude of EIMD (Fochi et al., 2016; Newham et al., 1988; Nosaka et al., 2005; Nosaka & Sakamoto, 2001; Váczi et al., 2009). Third, the total volume of work performed by the low-load group was notably greater than the high-load group (Nosaka et al., 2002$_b$). These factors may have confounded the EIMD response and masked the impact of exercise load.

Varied methods between trials were adopted by some studies included in this review, which could have confounded the impact of exercise load. For instance, providing a shorter inter-set rest period in the low-load compared with high-load exercise protocols was associated with more marked strength reductions <24 h post-exercise in some (Bartolomei et al., 2017; Nosaka et al., 2002$_b$) but not all (Draganidis et al., 2013; Paschalis et al., 2005; Peake et al., 2006) cases. Shorter inter-set rest periods can reduce total work capacity when exercise is performed to volitional failure (Evangelista et al., 2011). However, it is not clear whether the total work volume or number of sets performed influences exercise-induced strength loss. Between-group differences in strength decrements and recovery were noted both by Nosaka et al. (2002$_b$) and Chen et al. (2020), which might have been partly due to the varied number of sets performed between low- and high- load exercise conditions. Nonetheless, it was indicated by Barroso and colleagues (2011) that at a constant exercise intensity, the number of sets performed was not a determinant of exercise-induced strength loss. These conflicting data likely represent the impact of several exercise variables, not exercise load alone, on EIMD. Matching other exercise variables (joint starting angle, ROM, and contraction velocity) between conditions resulted in a greater immediate reduction in maximal arm strength following elbow flexor exercise performed at 110% compared to 70% of 1RM (Barroso et al., 2011). These data suggested that exercise load influences the magnitude of strength decline when other exercise variables are consistent between conditions. Therefore, standardising methods within studies and manipulating only exercise load is paramount to understanding its role in EIMD.

Disagreement regarding the impact of exercise load on strength loss may also be due to divergent methods used for assessing MVC, i.e. isotonic (Barroso et al., 2011; Draganidis et al., 2013) compared with isometric and isokinetic (Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002$_b$; Orssatto et al., 2018; Peake et al., 2006) strength tests. In support of this notion, both isometric and isokinetic knee flexor MVC were measured in trained males by Bartolomei and colleagues (2017) following back squat exercise performed at 70% or 90% of 1RM. Relative to with higher exercise loads, isokinetic MVC was reduced significantly more ≤24 h following exercise with lower loads, whereas the between-group difference in isometric

MVC was not evident until 48–72 h post-exercise. Likewise, between-group differences in the reduction in knee extensor (Orssatto et al., 2018) and elbow flexor (Peake et al., 2006) isometric MVC were apparent >24 h, but not immediately, post-exercise. These findings indicate a time-course discord between isometric and isokinetic strength reduction following resistance exercise. Therefore, comparing between studies that utilise varied methods to measure MVC may not be reasonable, and it follows that future studies should adopt standardised methods of strength assessment where feasible.

Muscle-damaging exercise protocols are often extreme and unrealistic of habitual training, and so, the outcomes may not be applicable outside of experimental settings. For example, untrained males were subjected by Chen et al. (2020) to 100 sets of 10 unilateral eccentric elbow flexions performed at 10% of MVC. Comparatively, only 12 sets of 10 high-load unilateral eccentric knee extensions were completed by untrained males previously (Paschalis et al., 2005), which was still considerably more than the recommended range of 1–3 sets for untrained individuals (ACSM, 2009). Extreme exercise protocols were indicated to impact the EIMD response to varied exercise load in the series of studies from Nosaka and colleagues. Unilateral elbow flexions were performed with a load equivalent to 9% of MVC, continuously for 2 h, by untrained males in their preliminary work (Nosaka et al., 2002_b). Significantly greater reductions in isometric MVC immediately post-exercise were induced by this protocol compared with the high-load protocol (12 sets × 1 maximal eccentric-only). In contrast, a low-load exercise protocol that reflected habitual resistance training (3 × 10 unilateral eccentric elbow flexions at 50% of isometric MVC) and was more akin to current training recommendations (ACSM, 2009) was utilised in their follow-up study (Nosaka & Newton, 2002). Opposing the previous findings (Nosaka et al., 2002_b), peak muscle force was better maintained at all post-exercise time-points with low-load relative to high-load (3 × 10 at 100% MVC) exercise. This study, as well as others (Arazi & Asadi, 2018; Orssatto et al., 2018), showed that real-life resistance exercise protocols can induce significant muscle damage and enable the detection of between-group differences; thus, are appropriate for use in experimental models of EIMD.

Overall, the studies reviewed here have indicated that resistance exercise load does not greatly influence early (<24 h) post-exercise reductions in MVC, as a similar number of studies reported comparable (n = 4) or greater preservation of MVC with low-load (n = 3) or high-load (n = 3) resistance exercise (*Figure 5.2*). During the later recovery period (24–96 h post-exercise), maximal strength appears to be more impaired following high-load resistance

exercise, implying a delayed recovery response relative to low-load exercise. Therefore, performing resistance exercise with lower loads may be advantageous first, to attenuate maximum strength decrements and second, to accelerate strength recovery.

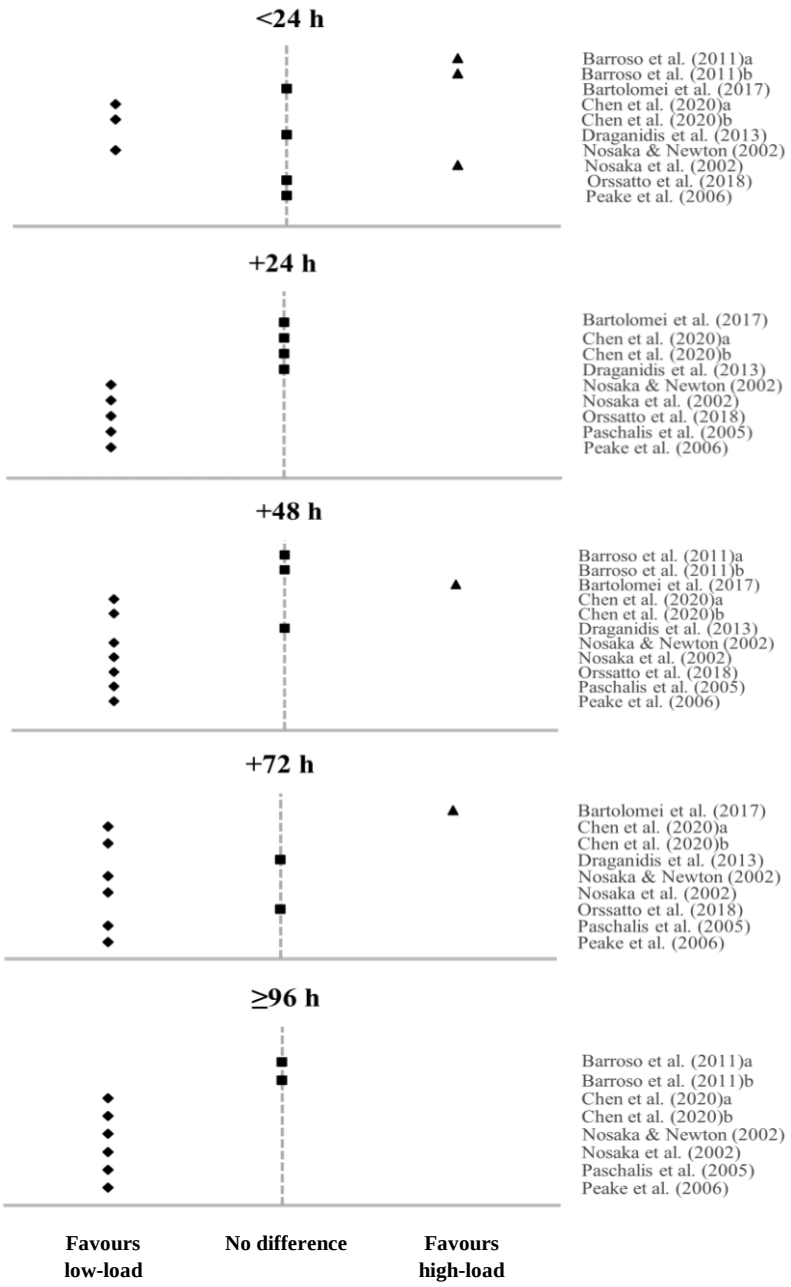


Figure 5.2. Overview of the exercise-induced change in maximal voluntary contraction among the studies reviewed.

Time-point is in relation to exercise; positioning in favour of exercise load represents a smaller reduction in MVC; subscripts a and b represent different trials within the same study.

5.4.3. The Impact of Exercise Load on Muscle Soreness

Muscle soreness was assessed in 11 studies (Arazi & Asadi, 2018; Barroso et al., 2011; Bartolomei et al., 2017; Chen et al., 2020; Draganidis et al., 2013; Hasenoehrl et al., 2017; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Paschalis et al., 2005; Peake et al., 2006; Uchida et al., 2009) all with use of VAS/VRSs and, in one study, pressure algometry (Peake et al., 2006). At 48 h post-exercise, when muscle soreness typically peaks (Clarkson & Hubal, 2002), 8 out of 16 trials reported significantly higher muscle soreness following high-load compared with low-load resistance exercise (*Figure 5.3)*. A negative impact of using lower exercise loads on muscle soreness was documented in only one trial (Bartolomei et al., 2017). Independent of exercise load, muscle soreness peaked at 24–48 h post-exercise (*Figures 5.4.a and b*), although tended to be alleviated more rapidly following low-load exercise (Chen et al., 2020; Draganidis et al., 2013; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Peake et al., 2006). Significant elevations in muscle soreness were sustained for 72–96 h post-exercise in both conditions in some studies (Arazi & Asadi, 2018; Paschalis et al., 2005), yet rarely did low-load (Barroso et al., 2011) or high-load (Bartolomei et al., 2017) exercise fail to induce significant increases in soreness.

Exercise-induced muscle soreness has been argued to poorly reflect the severity of EIMD (Nosaka et al., 2002_a) owing to its subjectivity and high variability (Damas et al., 2016_a). In support, varied changes in muscle soreness were identified in this review, with post-exercise increases ranging 10–61 and 22–70 mm (100 mm scale) following low- and high- load exercise, respectively. Muscle soreness variability appeared to be influenced by participant training status, irrespective of exercise load. Across all exercise conditions, peak muscle soreness ratings ranged 24–61 mm in resistance trained individuals and 15–80 mm in untrained individuals. Therefore, resistance exercise experience and familiarity with muscle soreness sensations may increase the accuracy in which muscle soreness rating scales are completed. Nonetheless, the impact of EIMD on muscle soreness is challenging to isolate, given that many other factors including present mood, hormonal status, and sleep quality (Lautenbacher et al., 2006; Melzack, 1982) can affect pain and soreness perception. Accordingly, subjective measures of muscle soreness in EIMD research should be interpreted with scepticism, as between- and within- group variability in muscle soreness outcomes may not necessarily indicate a treatment effect. Therefore, assessments of muscle soreness alone should not be used to determine the magnitude of EIMD in response to varied exercise load.

Low-load resistance exercise was rarely associated with more severe muscle soreness compared to high-load exercise in the current review. In one instance (Bartolomei et al., 2017), performing a high volume (80 total repetitions) of back squat exercise with a load equivalent to 70% of 1RM induced significant muscle soreness in trained males for up to 72 h post-exercise. Whereas, performing a lower volume (24 total repetitions) of exercise with a higher load (90% of 1RM) did not induce muscle soreness. Considering the exercise loads were similar and both rather high, it was feasible that differences in exercise volume (load × sets × repetitions) contributed to the contrasting muscle soreness responses between groups. In support, the exercise volume and inter-set rest period were standardised between groups in the tightly-controlled, five-arm study by Uchida et al. (2009) to examine the isolated impact of bench press load on muscle soreness. By this means, muscle soreness upon palpation was of equivalent magnitude after bench press exercise performed at 50%, 75%, or 90% of 1RM. While these data suggested that exercise load is not a determinant of muscle soreness, support from further, more robust experimental trials is needed.

Unlike previous observations (Uchida et al., 2009), it was indicated by the outcomes of this review that exercise load was a greater factor than exercise volume in muscle soreness. Performing unilateral eccentric elbow flexions with a lower load did not induce muscle soreness in untrained males, despite a greater total work volume to when higher loads were used (Barroso et al., 2011). The observation of attenuated muscle soreness has been supported by several others following high volumes of upper-body (Chen et al., 2020; Nosaka et al., 2002$_b$) and lower-body (Arazi & Asadi, 2018; Draganidis et al., 2013) low-load exercise, compared to lower volumes of exercise using heavier loads. Consequently, muscle soreness may be intensified by an increase in active muscle tension, as opposed to an increase in total muscle work output. As such, the utilisation of lower loads during resistance exercise could help manage muscle soreness even when high volumes of exercise are performed (Evangelista et al., 2011).

A benefit of using lower exercise loads for reducing muscle soreness was identified in half of the trials included in this review, while half reported no benefit (*Figure 5.3*). These conflicting data could be attributed to the varied methods used for assessing muscle soreness between studies. Muscle soreness assessments were conducted at rest (Barroso et al., 2011; Bartolomei et al., 2017; Hasenoehrl et al., 2017), during unloaded contraction (Arazi & Asadi, 2018; Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002$_b$), and during muscle palpation (Draganidis et al., 2013; Nosaka & Newton, 2002; Nosaka et al., 2002$_b$; Paschalis et al., 2005;

Peake et al., 2006; Uchida et al., 2009). Accordingly, lower muscle soreness in the low-load compared with high-load exercise group during passive contraction of the elbow flexors, but not during muscle palpation, was reported by Nosaka and colleagues (2002; 2002$_b$). Participants in the low-load group also experienced greater post-exercise elbow joint ROM, which suggested that muscle soreness related to muscle contraction might be a product of muscle stiffness; while muscle soreness associated with muscle palpation could be a consequence of oedema (Damas et al., 2016$_a$). Examining muscle soreness outcomes using different assessment methods would help establish whether disagreements among studies are due to inconsistent muscle soreness assessment methods. Further, this knowledge could help improve understanding of the mechanisms underpinning the development of exercise-induced muscle soreness.

Overall, it was discovered by this review that performing resistance exercise with lower loads typically induced a blunted and less sustained muscle soreness response relative to exercise with higher loads. Muscle soreness is a potential limiting factor of exercise compliance and future exercise quality and subsequently, exercise adaptations. Therefore, individuals may benefit from using lower resistance exercise loads to ensure repeated bouts of high-quality exercise can be performed without the hindrance of severe and sustained muscle soreness. All the while, the subjective and variable nature of muscle soreness, concurrent with its vulnerability to be affected by other physiological and psychological factors, should be considered when interpreting these outcomes.

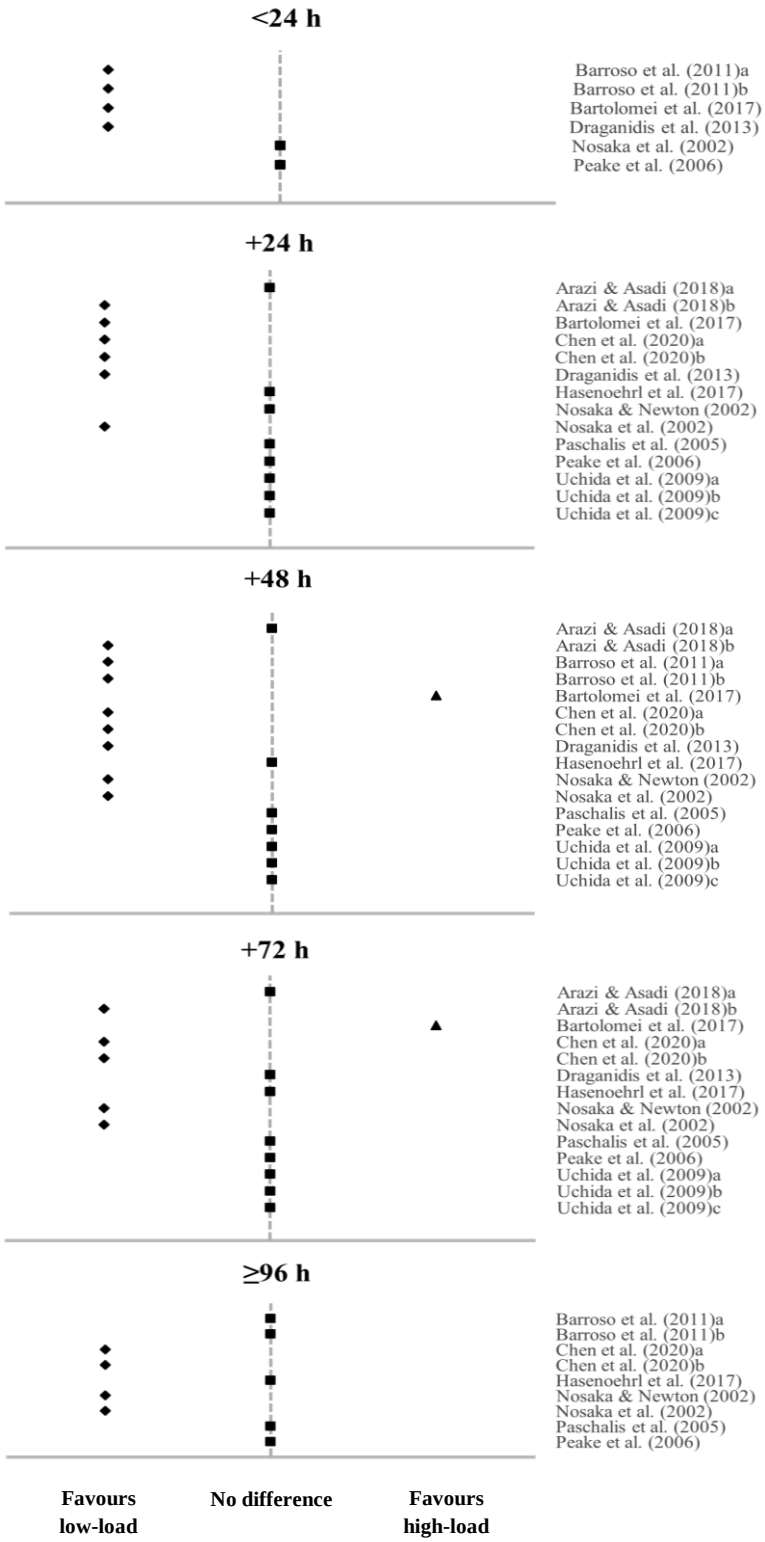


Figure 5.3. Overview of the exercise-induced change in muscle soreness among the studies reviewed.

Time-point is in relation to exercise; positioning in favour of exercise load represents a smaller increase in muscle soreness; subscripts a and b represent different trials within the same study.

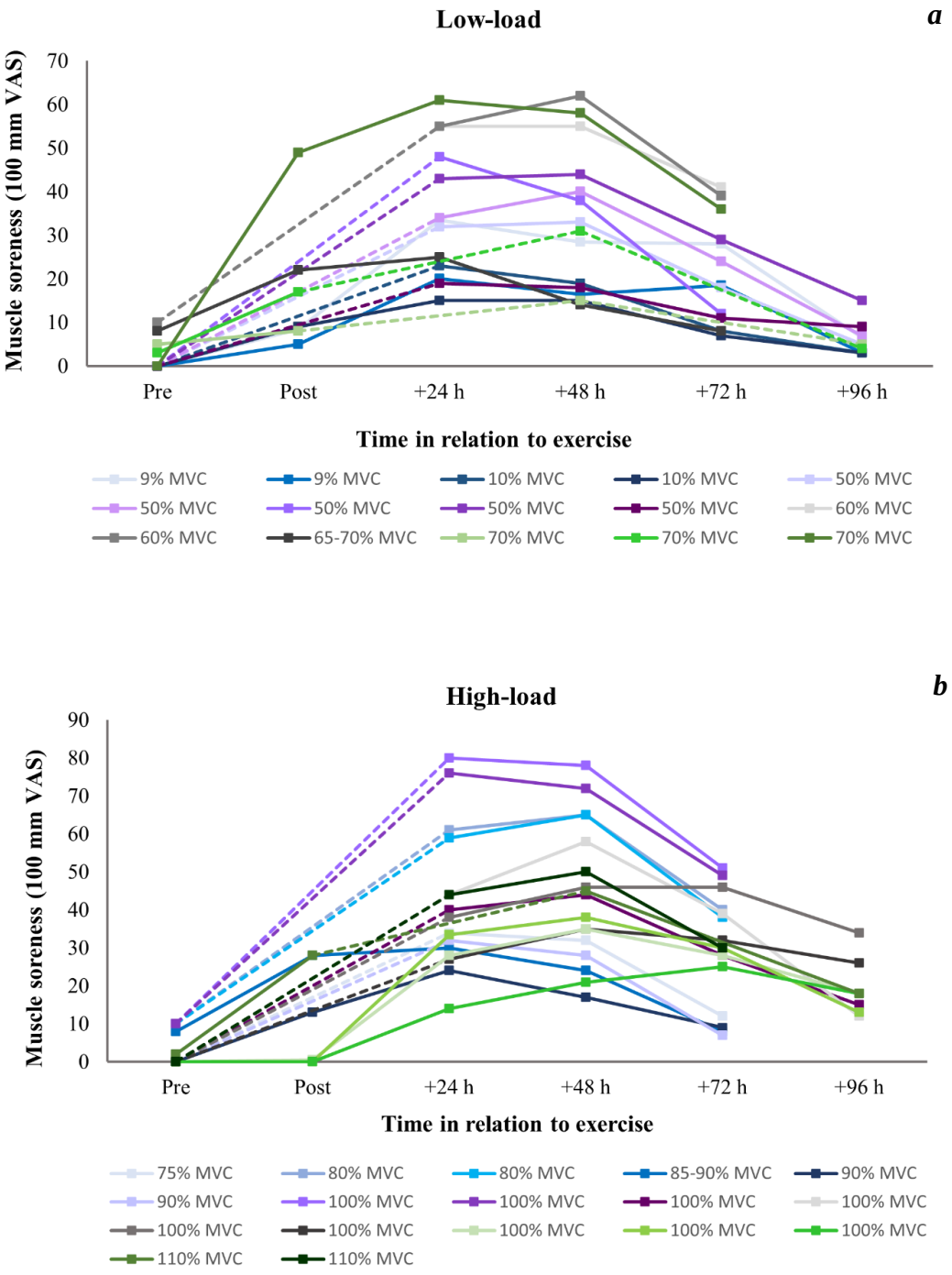


Figure 5.4. Time-course of muscle soreness from VAS ratings following a. low-load and b. high-load resistance exercise in the studies reviewed.

Dotted line signifies missing time-point. Data from: Arazi & Asadi (2018); Barroso et al. (2011); Bartolomei et al. (2017); Chen et al. (2020); Draganidis et al. (2013); Hasenoehrl et al. (2017); Nosaka & Newton (2002); Nosaka et al. (2002)$_b$; Peake et al. (2006); Uchida et al. (2009).

5.4.4. The Impact of Exercise Load on Blood Creatine Kinase Concentration

Creatine kinase concentration within plasma (Arazi & Asadi, 2018; Chen et al., 2020; Güzel et al., 2007; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Peake et al., 2006; Uchida et al., 2009) or serum (Bartolomei et al., 2017; Draganidis et al., 2013; Hasenoehrl et al., 2017; Mendham et al., 2011; Paschalis et al., 2005) was measured in 13 of the studies reviewed. Exercise-induced elevations in [CK] typically peak after 3–5 d (Clarkson & Hubal, 2002). Here, only 6 studies measured [CK] >72 h post-exercise, and therefore, many studies likely did not detect peak [CK]. As depicted in *Figure 5.5*, in no instance or time-point did low-load resistance exercise induce a significantly greater increase in [CK] than high-load exercise. The rise in [CK] at 48–72 h following low-load and high-load exercise was equal in 9 out of 15 trials.

Incrementally reducing resistance exercise load could have increasingly protective effects on cell membrane stability. As demonstrated by Arazi and Asadi (2018), leg press exercise performed at 80% 1RM by untrained males attenuated the CK response 24–72 h post-exercise compared to when performed at 100% 1RM. Lowering exercise load to 60% 1RM further attenuated [CK]. Likewise, following elbow flexions performed by untrained males, the [CK] rise was reduced with use of exercise loads equivalent to 10% of maximal relative to 50% and further abated relative to 100% (Chen et al., 2020). Taken together, these data suggested that higher resistance exercise loads – associated with greater muscle tension – disrupted myofibre membrane stability more so than lower exercise loads. Therefore, lowering active muscle tension by utilising exercise loads equivalent to 50–60% of 1RM could help maintain cell membrane permeability and reduce intramuscular protein leakage.

High intensity eccentric contractions are commonly believed to maximise muscle damage (Allen, 2001; Ebbeling & Clarkson, 1989; Margaritelis et al., 2021; Stauber, 1989). It was found by this review that eccentric exercise performed with heavier loads was associated with a greater rise in post-exercise [CK] in some (Chen et al., 2020; Nosaka & Newton, 2002; Paschalis et al., 2005) but not all (Hasenoehrl et al., 2017; Peake et al., 2006) studies. In a crossover design, [CK] comparably increased after eccentric elbow flexions performed to volitional failure at 50% or 100% of 1RM by trained males (Hasenoehrl et al., 2017). Similarly, 10 sets of unilateral eccentric elbow flexions completed at 10% or 100% of isometric MVC by untrained males significantly increased [CK] at 72–96 h post-exercise compared to baseline, with no differences between groups (Peake et al., 2006). Therefore, resistance exercise with a

largely eccentric component can cause significant muscle damage, evidenced by increased membrane permeability, irrespective of exercise load. It has recently been proposed that muscle damage induced by eccentric exercise is predominantly due to the unfamiliar nature of eccentric muscle contractions (Margaritelis et al., 2021). Accordingly, the EIMD response to eccentric resistance exercise of varied load may be influenced by training status. In support of this concept, similar increases in [CK] following supramaximal (110% 1RM) eccentric bench press exercise and submaximal (50%, 75%, or 90% 1RM) concentric bench press exercise performed by trained males were documented by Uchida and colleagues (2009). The training experience of these participants could have accustomed them to eccentric exercise and offered a protective effect against its damage, relative to those with little resistance training experience. This rationale (Margaritelis et al., 2021) might explain why utilising heavier loads during eccentric resistance exercise does not always cause greater muscle damage than when lighter loads are used.

Exercise-induced disruption to myofibre membrane stability seemingly depends on the muscle group exercised, with greater intramuscular protein leakage following exercise performed with upper-body muscle groups (elbow flexors and extensors) compared with lower-body muscle groups (knee flexors and extensors) (Chen et al., 2011). In the present review, peak increases from baseline in [CK] ranged ~113–400% and ~66–1600% after lower-body exercise (Arazi & Asadi, 2018; Bartolomei et al., 2017; Draganidis et al., 2013; Paschalis et al., 2005) and ~151–818% and ~75–5039% after upper-body exercise (Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Peake et al., 2006; Uchida et al., 2009) performed with low and high loads, respectively. Supporting previous observations (Chen et al., 2011), these data indicated that exercise-induced myofibre membrane disruption was greater when smaller muscle groups were engaged, irrespective of the exercise load.

In agreement with others (Nosaka & Clarkson, 1996_c), high variability in [CK] following low and high load resistance exercise was identified in this review (*Figures 5.6.a and b*). Individuals who experience robust exercise-induced increases in blood [CK] are considered 'high-responders' (Chen, 2006; Clarkson & Ebbeling, 1988). In the study by Nosaka and Newton (2002), untrained males presented low inter-individual variability in baseline [CK], yet post-exercise peak [CK] was highly variable among participants performing high-load (CV = 91%) and especially low-load (CV = 233%) resistance exercise. Likewise, CK responses to exercise were found by Peake et al. (2006) and others (Bartolomei et al., 2017) to be more variable when lower exercise loads were utilised [CV = 185% and 76% for low and high loads,

respectively; Peake et al. (2006)]. These studies suggested that exercise-induced [CK] variability was more pronounced when exercise was performed with lighter loads. Furthermore, it was highlighted by this review that individuals can be 'high responders' even when resistance exercise was performed with lighter loads.

Overall, the CK response to resistance exercise appears to be influenced by exercise load. Resistance exercise performed with low loads did not induce significantly greater elevations in blood [CK] compared to when performed with high loads in any study under review. However, in several cases, the CK response was comparable between low and high load exercise conditions, which may be attributed to other exercise variables or to individual variation in the CK response. Due to its variable nature and poor relationship with other muscle damage markers, it has been suggested that the analysis of [CK] and other blood proteins should not be used to assess EIMD; instead focus should be directed toward functional markers (Warren et al., 1999). This discord was evident in the present review, whereby significant differences between exercise conditions existed for changes in MVC or muscle soreness, but not for [CK] (Bartolomei et al., 2017; Draganidis et al., 2013; Peake et al., 2006) or vice versa (Paschalis et al., 2005). Also, from a practical perspective, [CK] measurements require advanced analytical techniques that are not feasible to the typical exerciser – unlike feelings of muscle soreness or weakness which can be easily gauged and used to judge when one is physically and mentally ready to resume exercise. Therefore, while examining CK responses to exercise has proven useful for understanding the mechanisms and management strategies for EIMD, other indirect muscle damage markers are perhaps more appropriate moving forward.

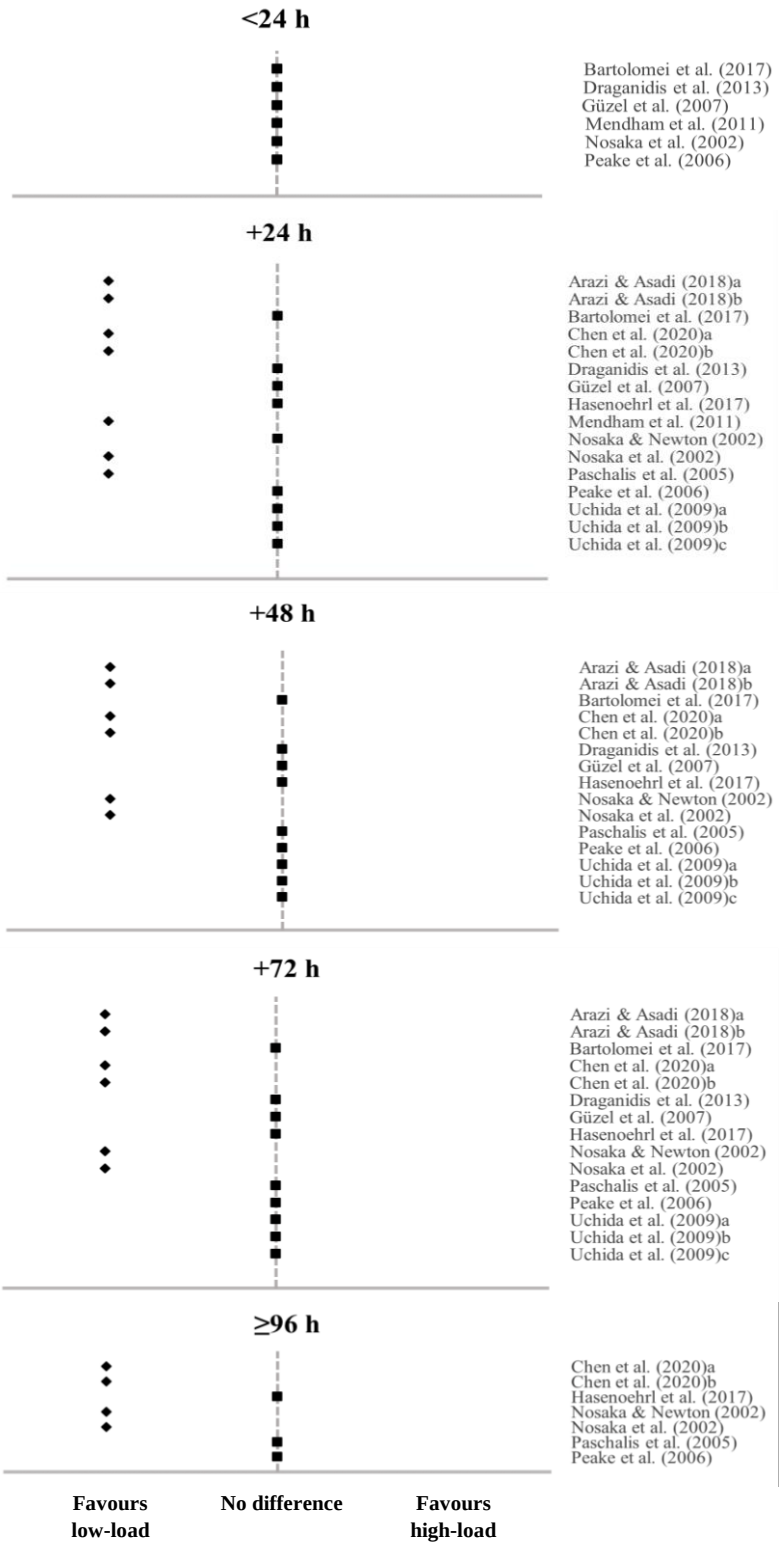


Figure 5.5. Overview of the exercise-induced change in creatine kinase concentration ([CK]) among the studies reviewed.

Time-point is in relation to exercise; positioning in favour of exercise load represents a smaller increase in [CK]; subscripts a, b, and c represent different trials within the same study.

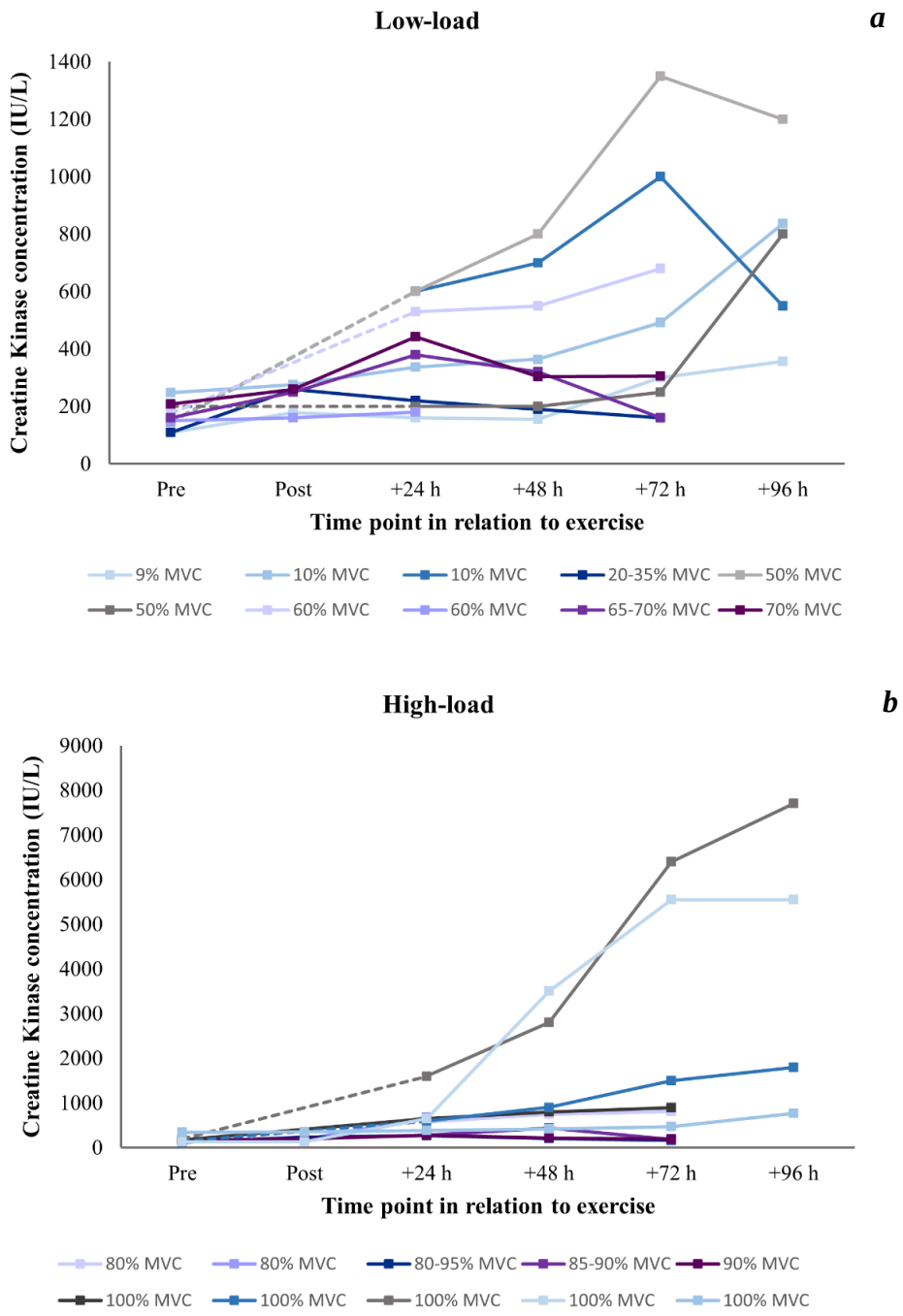


Figure 5.6. Time-course of change in blood creatine kinase concentration following a. low-load and b. high-load resistance exercise.

Dotted line signifies missing time-point; corresponding colours between figures 8.6.a and b. are from the same study. Data from: Arazi & Asadi (2018) ■; Bartolomei et al. (2017) ■; Chen et al. (2020) ■■; Draganidis et al. (2013) ■; Guzel et al. (2007) ■; Mendham et al. (2011) ■; Nosaka et al. (2002)b ■; Nosaka & Newton (2002) ■; Peake et al. (2006) ■.

5.5.0. Limitations and Directions for Future Research

There were several limitations to the current review that may impact the application of its outcomes. A key limitation was that the included studies encompassed a 98% male sample, and therefore, the outcomes may not be generalised to female populations. Further, most participants were young (<35 y), and so, the impact of exercise load on EIMD in older adults is inconclusive. Future EIMD research should include both male and female participants with the aim of comparing between sexes were feasible.

A crossover design was employed by many of the studies reviewed (Bartolomei et al., 2017; Cornish et al., 2018; Draganidis et al., 2013; Hasenoehrl et al., 2017; Mendham et al., 2011; Nosaka & Newton, 2002; Paschalis et al., 2005; Peake et al., 2006), which may have subjected these outcome data to RBEs (Hough, 1902; Nosaka & Aoki, 2011). The washout period between conditions (7–28 d) was generally insufficient to prevent the attenuation of muscle damage during repeated exercise bouts. Positively, a contralateral limb model was used in some studies to help manage RBEs (Nosaka & Newton, 2002; Paschalis et al., 2005). However, group allocation was not counterbalanced in some crossover studies, and so, these data may have been vulnerable to order-effects (Paschalis et al., 2005; Peake et al., 2006). Despite the high inter-individual variability of EIMD, between-subject parallel group study designs are more appropriate when RBEs effects cannot be adequately managed.

In many studies, the exercise protocols sought to maximise muscle damage, i.e. eccentric-only contractions of small muscle groups performed by untrained individuals (Barroso et al., 2011; Chen et al., 2020; Hasenoehrl et al., 2017; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Peake et al., 2006). Such experimental protocols do not reflect habitual exercise, which typically involves more than one muscle group and a combination of eccentric and concentric contractions. Studies that utilised more realistic exercise protocols in the present review sometimes (Arazi & Asadi, 2018; Bartolomei et al., 2017; Draganidis et al., 2013; Mendham et al., 2011; Orssatto et al., 2018) but not always (Cornish et al., 2018; Güzel et al., 2007; Uchida et al., 2009) reported an impact of exercise load on measures of EIMD. To better understand the relationship of exercise load and EIMD in a real-world setting, future research should use exercise protocols that mimic standard hypertrophy and strength training programmes.

Measures of muscle function, particularly MVC, have been deemed to best reflect the magnitude of EIMD (Damas et al., 2016_a). Several studies that solely measured the

concentration of blood proteins to assess EIMD were included in this review (Cornish et al., 2018; Güzel et al., 2007; Mendham et al., 2011), while others also included an assessment of muscle soreness (Arazi & Asadi, 2018; Uchida et al., 2009). These markers have shown to poorly relate to muscle function, and therefore, their use for EIMD assessments in the absence of functional measures has been discouraged (Nosaka et al., 2002_a; Warren et al., 1999). Only the change in MVC was examined as a functional marker of EIMD in the current review. Future research may wish to review the impact of exercise load on other functional markers, such as ROM and various exercise performance tests (power, speed, agility, sport-specific etc.), which could have wider application.

Another limitation of the present review was the thresholds used to classify exercise load (≤70% 1RM for low-load and >70% 1RM for high-load). Typically, exercise loads of ~70% 1RM would be deemed as high intensity. However, upon a preliminary study search, many trials compared exercise performed at 70% of maximal load against a higher intensity comparator. Therefore, exercise load was categorised as such to ensure maximal inclusion of relevant studies. Examining the impact of moderate exercise loads (possibly 50–70% 1RM) relative to low and high loads on EIMD may produce different outcomes.

5.6.0 Conclusion

This scoping review has demonstrated that resistance exercise performed with lighter compared to heavier loads often reduced muscle damage in adult males. In some cases, exercise load did not impact the severity of EIMD, though this might be explained by methodological differences within and between trials. As such, low-load resistance exercise is a viable management strategy for EIMD in males. It has already been established that resistance exercise performed with lower loads, while being perceptively less exerting, can induce comparable muscle adaptations to those resulting from exercise with higher loads. It follows that resistance exercise recommendations, particularly those targeted toward individuals who are novice, undergoing injury rehabilitation, older adults, or simply hesitant to utilise heavy weights, may wish to incorporate or exclusively use lighter weights. Due to a dominance of research conducted with males, the impact of exercise load on EIMD in females could not be concluded in the present review, and therefore, these recommendations might not be applicable to female populations.

5.7.0. Link to Next Chapter

It was identified by this scoping review that EIMD in males can be attenuated by utilising lower exercise loads. Given that the impact of exercise load on EIMD in females is currently unknown and may be subject to sex differences, it was considered important to conduct an RCT that included both sexes. Accordingly, a protocol for an RCT investigating the impact of low-load versus high-load habitual-type resistance exercise on EIMD responses in untrained males and females was described in the next chapter of this thesis.

CHAPTER 6 Sex differences in the impact of resistance exercise load on muscle damage: A protocol for a randomised parallel group trial

6.1.0. Prelude

While it was not possible to conduct this RCT during the course of this PhD, a modified version of the following study protocol has been published with open-access in PLOS One and may be used by researchers as a guide for developing similar studies. Focus was placed on involving appropriate female-orientated methodologies, for example, by controlling MC phase and analysing serum oestrogen concentration, allowing more accurate sex-comparison. This protocol incorporated the same EIMD markers and time-points of assessment as the RCT presented earlier in this thesis (*chapter 4*) to increase uniformity in EIMD research, which has often been lacking.

6.2.0. Introduction

Individuals engage in resistance exercise for a multitude of reasons, not limited to improving sport performance, enhancing body composition, delaying sarcopenia, and maintaining general health and psychological well-being. At a rudimentary level, repeated bouts of resistance exercise may culminate in skeletal muscle hypertrophy and increases in muscle force-generating capacity (Schoenfeld, 2010). Research over the past decade has challenged the notion that traditional high-load (>70% 1RM) resistance training is necessary to induce skeletal muscle adaptations (Schoenfeld et al., 2017_a). Training with exercise loads as low as 30% 1RM was suggested by Schoenfeld and colleagues (2017_a) to elicit comparable skeletal muscle hypertrophy and isometric strength gains to exercise with high loads. Nevertheless, the capacity of skeletal muscle to adapt to resistance training may be limited by acute muscle fibre damage (Damas et al., 2016_b).

Skeletal muscle damage is a potential consequence of unaccustomed or eccentrically-biased muscle contractions (Stauber, 1989). At a cellular level, the effects of the damage are targeted toward the structural components of sarcomeres and to the sarcolemma, resulting in the leakage of intramuscular proteins (e.g. creatine kinase and myoglobin) into systemic circulation (Allen et al., 2001; Morgan et al., 1996; Warren et al., 1993). However, of greatest deterrent to the exercising individual are those events that present themselves on the surface. Resistance EIMD may cause muscle soreness, limb swelling, reduced flexibility, and most notably, decreased muscle force-generating capacity (Clarkson & Hubal, 2002; Pyne, 1994; Warren et al., 1999). The presence of these impairments for up to a week following EIMD (Farup et al., 2014) increases the required recovery time preceding subsequent exercise bouts. In the absence of sufficient recovery, exercise performance may suffer and, by this means, restrict the occurrence of maximal training adaptations. Therefore, gaining understanding of the mechanisms underpinning EIMD and developing mitigation strategies is paramount to researchers and exercising individuals alike.

The severity of EIMD may be predetermined by several extrinsic variables of the exercise bout. These include the muscle group exercised (Chen et al., 2011), total number of eccentric contractions, velocity of movement (Chapman et al., 2008; Nogueira et al., 2013), muscle length at which the contraction is initiated and maximum force is generated (Nosaka et al., 2005; Váczi et al., 2009), and the maximum force produced (Nosaka & Newton, 2002). However, due to the inherent relationship between muscle force and length [i.e. length-tension

curve (Gordon et al., 1966)], it is challenging to ascertain whether the severity of EIMD is determined by the force production or length of the muscle, as they characteristically peak in unison. Therefore, it is important to understand the effects of these variables in isolation on EIMD. Studies in animals (Brooks et al., 1995; Talbot & Morgan, 1998) and humans (Fochi et al., 2016; Newham et al., 1988; Nosaka et al., 2005; Nosaka & Sakamoto, 2001; Váczi et al., 2009) have consistently demonstrated more severe EIMD when the targeted muscle was stretched over a greater range of muscle lengths. Conversely, when the maximum force output of the muscle is manipulated (i.e. by varying the exercise load or electrostimulation intensity) the impact on EIMD magnitude is less transparent, at least in humans. For example, of the studies comparing the impact of resistance exercise performed with low and high loads on various markers of muscle damage, several reported no differences between exercise loads for maximal voluntary contraction (Barroso et al., 2011; Draganidis et al., 2013; Uchida et al., 2009), subjective muscle soreness (Arazi & Asadi, 2018; Hasenoehrl et al., 2017; Paschalis et al., 2005; Peake et al., 2006; Uchida et al., 2009), or CK activity (Arazi & Asadi, 2018; Bartolomei et al., 2017; Draganidis et al., 2013; Güzel et al., 2007; Hasenoehrl et al., 2017; Paschalis et al., 2005; Peake et al., 2006). These data suggested that the muscle force generated during contraction was not necessarily a prerequisite to EIMD. Although, in the instances whereby EIMD differed between exercise loads, the outcome measures predominantly favoured the low-load condition (Chen et al., 2020; Nosaka & Newton, 2002; Nosaka et al., 2002_b; Orssatto et al., 2018). In this regard, performing resistance exercise with lighter loads may be a viable alternative to traditional high-load resistance exercise to attenuate EIMD, without dampening muscular adaptations to training.

The current literature surrounding the EIMD response to varied exercise loads has underrepresented exclusively female populations. Combined data from older male and female adults was reported by Orssatto and colleagues (2018), in which resistance exercise performed at 85% relative to 60% 1RM significantly reduced maximal isometric torque of the knee extensor and flexor muscles. Young females alone were studied by Alvarez and colleagues (2020), yet the exercise protocol incorporated blood flow restriction in the low-load condition only, which was associated with mild muscle soreness. Therefore, the impact of exercise load on EIMD is poorly understood in females.

The question as to whether EIMD is sex-specific is heavily debated among researchers (Hubal & Clarkson, 2009; Pizza, 2009; Tiidus & Enns, 2009). Fundamental physiological differences between males and females, including but not limited to skeletal muscle fibre type distribution,

body adiposity, bone mineral density, maximal strength, muscle fatigue resistance, and sex hormone milieu (Ansdell et al., 2020; Ivey et al., 2000; Karastergiou et al., 2012; Makovey et al., 2005; Miller et al., 1993; Staron et al., 2000; Taraborrelli, 2015) may cause disparate EIMD responses between sexes. While early animal models have strongly supported a role of oestrogens in protecting against EIMD (Amelink & Bär, 1986; Amelink et al., 1990; Bär et al., 1988; Clarkson & Sayers, 1999; Komulainen et al., 1999; St. Pierre Schneider et al., 1999), this argument has not held in humans. Following eccentric exercise, females have experienced more severe strength impairments (Minahan et al., 2015; Sewright et al., 2008), inflammation (MacIntyre et al., 2000), and intramuscular protein leakage (Fredsted et al., 2008) relative to their male counterparts. Consistent with previous animal models, attenuated EIMD in females has been reported in some studies (Fernandez-Gonzalo et al., 2014; Kerksick et al., 2008; Stupka et al., 2000), whereas others have failed to identify sex differences (Dannecker et al., 2012; Eston et al., 2000; Hubal et al., 2008; MacIntyre et al., 2000; Morawetz et al., 2020; Rinard et al., 2000; Sayers & Clarkson, 2001). Furthermore, the findings from studies investigating the effects of HRT (Dieli-Conwright et al., 2009), HC use (Carter et al., 2001; Minahan et al., 2015), and MC phase (Romero-Parra et al., 2021_a; 2021_b) have been ambivalent. The determination of sex-dependent responses to EIMD is warranted to better understand the factors influencing and potential management strategies for EIMD.

The following protocol is designed to address two aims: first, to investigate the acute muscle damage response to a single bout of resistance exercise performed with low or high loads; and second, to compare the EIMD response between untrained male and female young adults. Combined with the growing body of literature, the outcomes of this RCT would inform the development of sex-specific training recommendations to maximise adaptations while minimising muscle damage. This protocol may be replicated or used as a basis for future research on sex differences in EIMD.

6.3.0. Methods

6.3.1. Study Design

A randomised, parallel group design will be employed to examine sex differences in indirect markers of muscle damage in response to low-load and high-load resistance exercise. Following an initial virtual screening to assess participant eligibility, the first two laboratory visits will act as familiarisation sessions, during which leg muscle function and body composition will be assessed. Participants will then be stratified by sex and randomised to either a low-load (30% 1RM) or high-load (80% 1RM) exercise condition. A three-week period will separate the familiarisation visits and the muscle damage exercise protocol to reduce the influence of RBEs and to allow all measures to be conducted during the late follicular phase of the MC in female participants. Venous blood samples and measures of limb circumference, ROM, muscle soreness, and PPT will be obtained before, immediately after, and 4, 24, 48, 72, and 168 h after the exercise bout. These time points should allow the peak values for each marker to be captured, which are expected to progressively return to baseline values by 168 h post-exercise. Additionally, during the 72 and 168 h post-exercise visits, assessments of muscle function (leg peak torque, countermovement jump height, and 30-m sprint) will be repeated. Muscle function will not be assessed immediately, 24, and 48 h post-exercise to mimic a realistic training schedule in which a muscle group would be rested for ≥2 d. A schematic overview of the study design is presented in *Figure 6.1*

6.3.2. Participants and Recruitment

Forty-eight healthy participants (24 males and 24 females, aged 18–35 years) will be recruited to participate in this study. Participants will not have performed regular (≥twice weekly) resistance or eccentrically-biased exercise during the previous six-month period. Participants will be recruited primarily from the Durham University student and staff cohort using a convenience sampling strategy with sex stratification. Prospective participants will be invited to volunteer for the study via email and social media (Twitter, LinkedIn) advertisements, as well as word-of-mouth. A statistical power analysis was conducted using G*Power 3.1 to determine the study sample size. The power calculation was based on a similar study by Orssatto et al. (2018), which reported a significant difference in the change in isometric MVC from baseline to 24 h post-exercise between low-load and high-load groups of 6.5%. The post-exercise change in MVC is deemed the most valid indirect indicator of EIMD (Damas et al., 2016$_a$). The calculation revealed that 12 participants per group (48 total) were required to have

80% power to detect a significant difference between groups when using a dependent *t* test with a 0.05 two-sided significance level. Therefore, a total of 56 participants (14 low-load male, 14 low-load female, 14 high-load male, 14 high-load female) will be recruited to allow for 20% drop-out.

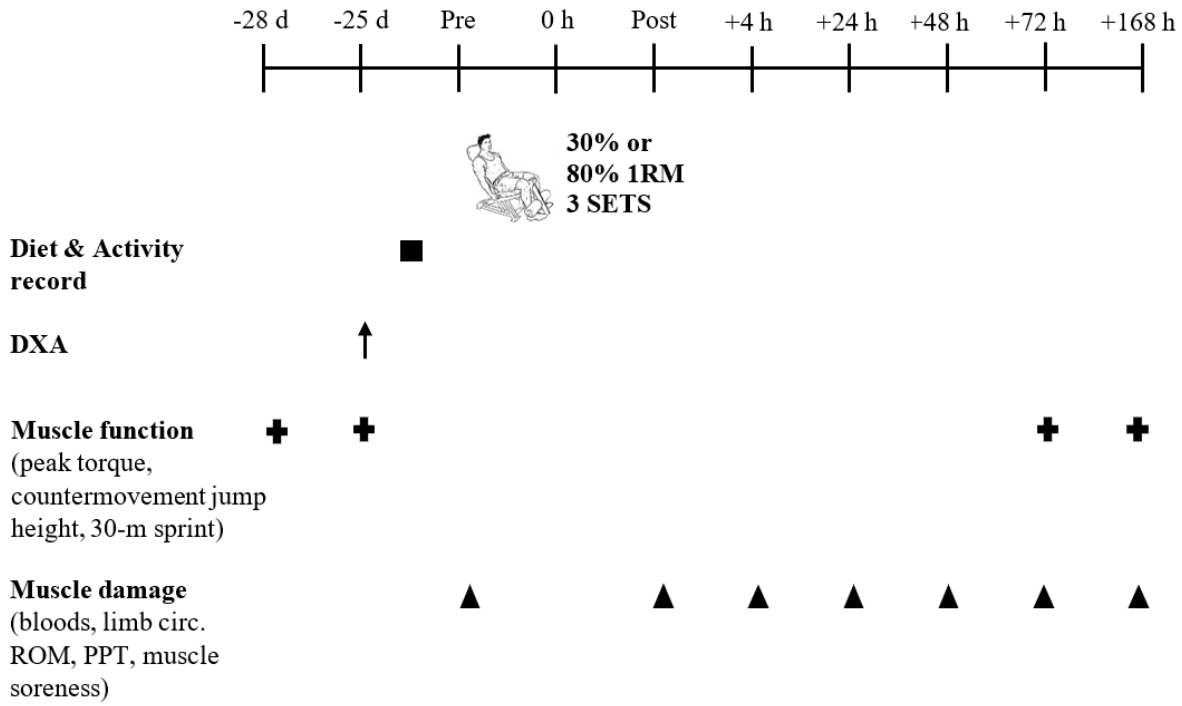


Figure 6.1. Study design.

DXA = dual-energy X-ray absorptiometry; 1RM = one repetition maximum; limb circ. = limb circumference; ROM = range of movement; PPT = pressure-pain threshold.

6.3.3. Inclusion Criteria

The study will include male and female adults who:

1. Are aged 18–35 years old
2. Are a healthy weight (BMI >18.5 and <25 kg/m^2)
3. Have no recent (previous 6 months) experience in resistance exercise
4. Are able to perform leg-based resistance exercise
5. Are free from musculoskeletal injury (past 6 months)
6. Are eumenorrheic (females only; regular MC over the past 12 months)

6.3.4. Exclusion Criteria

The study will not include participants who:

1. Habitually perform resistance exercise or eccentrically-biased exercise (e.g. downhill running)
2. Habitually (i.e. twice per week for previous 1-month period) consume ergogenic aids (e.g. creatine monohydrate), protein-based supplements, non-steroidal anti-inflammatory drugs, or nutritional supplements that may alleviate muscle damage (e.g. antioxidants, polyphenols, omega-3 polyunsaturated fatty acids)
3. Frequently engage in massage, cryotherapy, or other physiotherapeutic aids
4. Have a chronic disease or acute illness
5. Are pregnant or breast-feeding
6. Are smokers

6.3.5. Ethical Approval

This study protocol has received approval by the Department of Sport and Exercise Sciences Research Ethics Committee at Durham University (SPORT-2020-11-14T11_22_22-vlcz52; May 2021) and the Tyne and Wear South NHS Research Ethics Committee (21/NE/0073; May 2021). Informed written consent will be obtained from participants prior to any experimental procedures. This study has been registered at ClinicalTrials.gov (ID: NCT05111054).

6.3.6. Randomisation

Following an initial virtual screening questionnaire and baseline assessments, participants will be randomised to an experimental condition (low-load or high-load resistance exercise). Folded pieces of paper labelled either 'LL' (low-load) or 'HL' (high-load) will be placed into an opaque envelope, and one will be drawn by the participant to determine their exercise condition. To ensure an equal number of males and females in each group, separate envelopes will be used, i.e. the 'male' envelope will contain 12 papers labelled 'LL' and 12 'HL', as will the 'female' envelope. Due to the nature of the trial (i.e. varied intensity resistance exercise) it is not possible to blind the participant nor researcher to the experimental conditions.

6.3.7. Baseline Assessments and Familiarisation

After the initial virtual screening, female participants will be asked to record their MC and inform the lead researcher at the onset of menses. This information will be used to estimate the timing of the late follicular phase of the MC, during which all baseline and experimental measures will be conducted.

During the first laboratory visit (-28 d), participants will arrive in the morning in a post-prandial state and complete a standard health and readiness to exercise questionnaire. Next, participants will be demonstrated the correct use of the isokinetic dynamometer, with which the assessment of leg peak torque will be completed, as previously described (Gentil et al., 2017). Briefly, the peak torque of the knee extensors and flexors will be measured on the dominant and non-dominant leg in the same order for all participants. Prior to testing, the dynamometer's calibration will be completed as per the manufacturer's instructions. The participant will be seated with the dynamometer arm rotation axis aligned with the lateral condyle of the knee. The thigh, pelvis, and trunk will be secured to the dynamometer chair using belts to limit movement, and the positioning will be recorded and maintained for each assessment. For the tests, two sets of four repetitions will be performed at 60 º/s with a 1-min inter-set rest period. Participants will be instructed to fully extend/flex the knee joint at maximum intensity during each repetition, and the highest value will be regarded as the peak torque. Next, countermovement jump height will be assessed. Participants will be instructed to stand on a force plate, squat down, and maximally jump vertically, with their hands remaining on their hips. Participants will complete three countermovement jumps with a 1-min rest between each attempt, and the maximal height will be used for analysis. Finally, the 30-m sprint assessment will be conducted. Participants will be asked to stand 30 cm behind the timing gates, from which the sprint will be initiated. Participants will perform a single, maximal effort 30-m sprint, and the sprint time will be used for analysis.

Three days later (-25 d), participants will return to the laboratory in the morning following an overnight fast and abstinence from vigorous exercise and alcohol during the prior 24 h. Firstly, body mass and stature measurements will be obtained in light clothing. Female participants will confirm the absence of pregnancy, then whole-body composition will be assessed using DXA (GE Lunar iDXA, GE Healthcare, Madison, WI). Participants will be asked to consume 500 mL of water upon waking to ensure a euhydrated state and to wear minimal and metal-free clothing (e.g. t-shirt and pair of shorts) during the scan, in accordance with the best practice procedures for DXA measurement (Nana et al., 2015; Rodriguez-Sanchez & Galloway, 2015). Total body mass, LBM, fat mass, and BF% will be measured. Following the assessment of body composition, the peak torque assessment will be repeated to confirm the participants' peak torque.

6.3.8. Activity and Dietary Control

During the 3 wk rest period that will follow baseline measures, participants will be instructed to record their habitual activity and dietary intake for 3 d. This information will be used to examine any between-group differences. For each subsequent visit during the experimental period (+0, +24, +48, +72, and +168 h), participants will be provided with a standardised breakfast (125 g low-fat fruit yoghurt, 2 slices of wholemeal bread/toast with jam/honey, and 200 mL semi-skimmed milk; 443 kcal, 20 g protein, 73 g carbohydrate, 7 g fat) to consume at home ~3 h prior to attending the laboratory. Otherwise, participants will consume their habitual diet, and a 24 h dietary recall will be conducted during each visit to identify any within- and between- group differences. Participants will be instructed to abstain from the use of non-steroidal anti-inflammatory drugs, strenuous exercise, engagement in massage or cryotherapy, and the consumption of alcohol, protein supplements, anti-oxidant vitamins, omega-3 polyunsaturated fatty acids, and ergogenic aids during the study period. All dietary intake data will be analysed using Nutritics software (NutriticsLTD, Swords, Ireland).

6.3.9. Resistance Exercise Protocol

All resistance exercise sessions will be supervised. The described protocol will be performed on the leg extension machine followed by the leg curl machine (Versa leg extension/leg curl, Matrix, Wisconsin, USA), separated by 5 min rest. First, the correct form for use of the leg extension and leg curl resistance exercise machines will be demonstrated to the participants. Next, participants will perform a warm-up set consisting of 10 repetitions at 50% of their pre-determined 1RM. After 2 min rest, participants will complete 3 sets at their allocated exercise load (30% or 80% 1RM) each performed to volitional failure (defined as when a full repetition cannot be completed). A rest period of 2 min will be awarded between sets. A lifting tempo target of 1 and 2 s for concentric and eccentric phases of muscle contraction, respectively, will be set. Strong verbal encouragement will be given to all participants during exercise to ensure volitional failure is achieved.

6.3.10. Muscle Damage Markers

The following assessments of muscle damage will be conducted in the same order during each visit (pre, post, 4, 24, 48, 72, 168 h post-exercise) for all participants.

Blood sampling. Blood samples will be collected from an antecubital vein of the forearm using standard venepuncture techniques into three reagent-free vacutainers (3 × 10 mL). Samples will be left at room temperature for 30 min before being stored on ice and will later be analysed for serum concentrations of CK, IL-6, and oestrogen. Peak elevations in [CK] – an

intramuscular enzyme – are typically measured 2–5 d following EIMD and indicate an increase in sarcolemma permeability. IL-6 is a pro-inflammatory cytokine and its elevated serum concentration within proximity to exercise (0–6 h) suggests acute inflammation and marks the secondary muscle damage response. Oestrogen may have a protective effect against EIMD, although the evidence in humans is not substantial.

Limb circumference. An increase in limb circumference following exercise is indicative of muscle swelling and may be used as a proxy measure of inflammation. Participants' limb circumference will be measured using a standard anthropometric measuring tape at the mid, lower-, and upper- quartile points of the trochanterion-tibiale lateral site with the participant in a standing position. The mean value of the 3 sites will be used for the analysis.

Range of motion. Participants' ROM of the knee joint will be calculated as the difference between the relaxed and flexed knee joint angle, as measured using a standard goniometer with the participant in a supine position.

Muscle soreness. Participants will first rate their muscle soreness using a 10-point VRS ranging from 'not sore at all' to 'extremely sore' while performing a simple bodyweight squat. Secondly, muscle soreness will be assessed with the PPT test using a computerised pressure algometer (Medoc, AlgoMed, Ramat Yishai, Israel) with the participant in a supine position. The probe head (1 cm^2) of the algometer will be placed at the mid, lower-, and upper- quartile points of the trochanterion-tibiale lateral site and increasing pressure will be applied until the participant indicated pain. The mean value of the 3 sites will be used for the analysis.

Peak torque, countermovement jump height, and 30-m sprint. These assessments of muscle function will be conducted in the same order for each participant at 72 and 168 h post-exercise.

6.3.11. Serum Preparation and Analyses

Whole-blood samples will be centrifuged at 4 °C with 1100 g force for 15 min within 2 h of collection. Serum samples will be transferred into 1.5 mL microcentrifuge tubes and stored at -80 °C until subsequent analysis. The concentrations of CK, IL-6, IL-10, TNF-a, myoglobin, C-reactive protein, skeletal troponin I, myosin heavy chain fragments, and oestrogen within serum will be determined with commercially available assay kits.

6.3.12. Statistical Analyses

Statistical analysis will be conducted using IBM SPSS (version 25, SPSS Inc., Chicago, IL). All assumptions for statistical models will be assessed using the Shapiro–Wilk test, and data that violate the assumptions will be log transformed prior to analysis. Independent *t*-tests with Bonferroni corrections will be used to examine any between-group differences in baseline variables. The Levene's test will be used to check for equality of variances between groups. A two-way mixed design analysis of variance will be used to analyse all muscle damage markers and dietary intake data between exercise conditions (low-load male, low-load female, high-load male, and high-load female) and within time points (-28 d, -25 d, pre, post, +4, +24, +48, +72, and +168 h). Data sphericity will be assessed with the Mauchly's test and any data that violates the Greenhouse–Geisser assumptions will be corrected with Huynh–Feldt. Any significant group × time interactions will be analysed *post hoc* using independent *t*-tests with Bonferroni corrections for between-group comparisons at each time point. Within-group differences across time will be analysed using paired *t*-tests. The within-group mean change from baseline to each time-point will be reported for each muscle damage marker to allow for future calculation of ESs and inclusion in meta-analyses. Stepwise multiple regression analyses will be conducted with age, sex, exercise intensity, HC use, habitual activity, habitual daily protein intake, and body composition outcomes as the independent variables to identify predictors and confounders of each muscle damage marker. Statistical significance will be set at $P < 0.05$. Confidence intervals assume 95% confidence in the range of the mean. All data will be reported as mean ± SD unless otherwise stated.

6.3.13. Data storage and Dissemination of Findings

To ensure data protection, digital participant information will be stored on a password protected computer and data collection papers will be stored in a locked filing cabinet at the study site, available only to the primary investigators. Participant anonymity will be maintained through use of coded identification numbers, and all identifiable data will be destroyed following analysis of the complete dataset. Anonymised data will be uploaded to the institution's repository to be available for secondary analysis. The study findings will be disseminated by publications in peer-reviewed journals and conferences.

6.4.0. Discussion

This paper presents the study protocol for a randomised parallel-groups trial examining the impact of resistance exercise load on various indirect markers of muscle damage in untrained male and female young adults. The study protocol held the aim of establishing whether the acute muscle damage response to a single bout of resistance exercise was comparable between first, low and high exercise loads, and second, males and females. Key aspects of the target population, exercise protocol, muscle damage markers, and time-points of assessment have been detailed. Equivocal findings regarding the impact of resistance exercise load on EIMD have been produced by previous reports, which may partly be due to inter-study differences in methodological design. Nonetheless, no study has yet considered the influence of sex. The proposed study aims to provide a direct sex comparison of muscle damage responses to low-load and high-load resistance exercise. It was hypothesised that the high-load resistance exercise will induce more marked changes in muscle damage markers both in males and females, though it cannot be ascertained at this time whether sex differences will be present. The outcomes of this study may inform the development of sex-specific training recommendations to maximise exercise adaptations while minimising muscle damage.

Advancing this work could further inform exercise training recommendations by expanding the study outcomes and considering alternative target populations and exercise protocols. For example, we have proposed that muscle swelling – an indicator of inflammation – be conveniently assessed via limb circumference measurements. However, if available, more advanced techniques for assessing muscle swelling e.g. ultrasound imaging would provide additional information on muscle cross-sectional area, thickness, volume, pennation angle, and fascicle length. Furthermore, we have suggested that leg-based resistance exercise be performed to induce muscle damage, though alternatively, the impact of upper- or whole- body resistance exercise with varied loads could be explored. Following EIMD, the present protocol excludes the measurement of peak torque and other muscle function outcomes during the early post-exercise recovery period. This was suggested to avoid further muscle damage being induced by the muscle function tests and to better reflect real-life exercise practice, in which a day or two of rest would ensue the initial exercise bout. However, it was appreciated that peak torque is most impaired immediately following a damaging exercise bout, and thus, this time-point may wish to be captured.

Finally, the application of this research to wider populations could be achieved through its replication in diverse study samples. Conducting this research in untrained male and female young adults would be a pragmatic starting point, though comparing between male and female older adults or between trained and untrained females is suggested as future direction. The present research protocol may be used to guide the design of future studies aiming to bridge the sex data gap and inform sex-specific exercise recommendations.

7.1.0. Thesis Outcomes

The aim of this research thesis was to improve understanding of the contributing factors and management strategies for resistance EIMD, with an overarching aim of identifying and bridging the sex data gap in EIMD research. The following questions were addressed to achieve this aim:

1) What is the current evidence regarding the efficacy of dietary protein as a management strategy for resistance exercise-induced muscle damage? (*Chapter 3*)
2) Does the consumption of milk protein impact resistance exercise-induced mild muscle damage and does this response differ between untrained males and females? (*Chapter 4*)
3) Does the total and regional body composition of untrained males and females relate to resistance exercise-induced muscle damage? (*Chapter 4*)
4) Is performing low-load resistance exercise an effective strategy for reducing exercise-induced muscle damage? (*Chapter 5*)

A summary of the main outcomes of this thesis have been presented here and discussed within each experimental chapter:

1) Peri-exercise protein supplementation appeared beneficial for managing some, but not all, symptoms of resistance EIMD in young males. There is a paucity of data from females and so outcomes may not be extrapolated between sexes.
2) Peri-exercise milk protein ingestion did not reduce muscle damage or accelerate recovery following resistance exercise in untrained young adults, though females experienced attenuated EIMD relative to males.
3) Total and regional body composition outcomes were negatively associated with post-exercise CK responses in females, but not males.
4) Utilising lower resistance exercise loads was beneficial for attenuating EIMD and accelerating exercise recovery in males, though the response in females is currently unknown.

7.1.1. Management Strategies for EIMD

EIMD poses a threat to exercising individuals at recreational through to elite levels. Recreational exercisers especially could benefit from EIMD management strategies; particularly those targeted toward reducing perceptual symptoms, notably muscle soreness, owing to the negative impact that perceived soreness sensations may have on exercise adherence. Maintaining exercise adherence in recreational exercisers is imperative for achieving the health benefits and muscle adaptation associated with regular exercise. Whereas, for competitive or elite athletes, such symptoms of muscle soreness, stiffness, and weakness might not necessarily hinder exercise adherence because athletes may be more inclined to 'push through the pain' compared with recreational exercisers, given that high volumes and intensities of training are a prerequisite for their athletic success. Therefore, the original elements of this thesis focused on EIMD in individuals untrained in resistance exercise.

The relevance of EIMD in the context of muscle adaptation to exercise is still debated. Some have argued that post-exercise muscle damage and inflammatory responses are required for muscle adaptation to exercise (Chazaud, 2016; Damas et al., 2018), while others argued the contrary (Damas et al., 2016_b; Eriksson et al., 2006; Foley et al., 1999; Lauritzen et al., 2009). However, longitudinal changes in muscle mass and strength during resistance training – especially progressive resistance training – while sporadically assessing acute muscle damage have been examined in few studies (Lockwood et al., 2017; Roth et al., 1999; 2000_a). The outcomes of these studies implied that adaptation to exercise was not impeded by muscle damage and that muscle damage was not necessary for adaptation to occur. Further studies of this nature would help confirm the interplay between EIMD and adaptation. Until these are completed, it is perhaps pragmatic to strategically manage EIMD such that damage is not so severe that muscle adaptation to exercise is hindered, while ensuring the stress is sufficient to trigger adaptation.

Exercise protocols designed to induce severe muscle damage, for example by incorporating high volumes of eccentrically-biased contractions, have generally been utilised in previous experimental models of EIMD. However, these exercise protocols lack ecological validity. By means of using an exercise protocol that mimics habitual resistance training practices, this thesis has demonstrated that a physiologically-relevant exercise stimulus induces mild muscle damage, while maintaining maximal strength (*chapter 4*). Accordingly, exercising individuals in a real-world setting will likely not experience the severity of muscle damage incurred in

experimental models of EIMD. Similar exercise protocols have been implemented in longitudinal training studies and have exhibited success for inducing muscle hypertrophy (Aagaard et al., 1996; Fisher & Steele, 2017; Mitchell et al., 2012). The outcomes of *chapter 4*, together with previous studies (Roth et al., 1999; 2000_a), suggested that mild muscle damage does not hinder muscle adaptation to exercise, and severe muscle damage is not a prerequisite for adaptation. Establishing management strategies for mild EIMD is logically the next research avenue to explore.

To manage symptoms of EIMD, favourable strategies that include cryotherapy, vascular occlusion, compression garments, and various nutritional strategies, have been identified by prior research. This thesis demonstrated that, overall, peri-exercise protein supplementation was beneficial for the symptom management of EIMD in young males (*chapter 3*). Specifically, various protein supplementation protocols (different sources, doses, frequencies, timings, and durations of intake) implemented around acute resistance exercise attenuated post-exercise maximal strength decrements and blood [CK] elevations, but not perceived muscle soreness. These outcomes agree with previous works that reported an advantage of supplemental whey protein for restoring muscle function after resistance exercise (Davies et al., 2018), but minimal benefit of protein or amino acid supplementation for attenuating post-exercise muscle soreness (Pasiakos et al., 2014). In contrast to the outcomes of *chapter 3*, Pasiakos et al. (2014) found minimal evidence for a benefit of protein or amino acid supplementation for restoring muscle function or lessening the [CK] rise after resistance or endurance exercise. This thesis has added to these works by utilising more stringent study inclusion criteria, such that the outcomes relate specifically to muscle damage induced by resistance exercise with protein feeding, which likely explains the differing outcomes of these reviews.

The current review (*chapter 3*) examined the impact of various ingested protein sources, such as whey, soy, pea, egg white, and milk, on EIMD. Current nutritional guidelines advocate a food-first approach and to this end, it is of interest to examine whole-food sources of protein in the context of EIMD. Whole-food sources of milk protein, including dairy milk, yoghurt, and cheese, are of particular interest given they are an existing dietary staple, and their consumption peri-exercise is encouraged to stimulate increased rates of MPS and, in the case of fluid milk, muscle glycogen resynthesis to aid exercise recovery (Ferguson-Stegall et al., 2011; Wilkinson et al., 2007). However, in disagreement with others (Cockburn et al., 2008; 2010; 2012; Draganidis et al., 2017; Hirose et al., 2013) it was demonstrated by this thesis that

milk protein was not an effective strategy for EIMD management in males or females (*chapter 4*). In this RCT, dairy yoghurt was ingested in 4, evenly distributed, daily doses each providing 20 g of milk protein. This protein supplementation protocol was based on the premise that 20 g of milk protein (whey) maximally stimulated MPS rates following leg-only resistance exercise (Moore et al., 2009; Witard et al., 2014) and this daily distribution pattern is anabolically preferred over bolus (2 × 40 g) or pulse (8 × 10 g) protein feeding patterns in young males (Areta et al., 2013). The regular consumption of whole-food milk products providing ~20-g protein doses in combination with resistance training promotes favourable changes in body composition, skeletal muscle hypertrophy, and strength gains over time (Bridge et al., 2019; Hartman et al., 2007; Josse et al., 2009). However, regularly ingesting 20-g doses of milk protein did not attenuate acute muscle damage or accelerate recovery following leg-based resistance exercise relative to an isoenergetic non-dairy placebo product in the current research (*chapter 4*). The protein dose provided was likely sufficient to stimulate post-exercise MPS, considering that the exercise volume performed was lower than in Witard et al. (2014) who identified that a 20-g protein dose was optimal for MPS stimulation after leg exercise. Furthermore, it was demonstrated by Cockburn et al. (2012) that higher (34 g) and lower (17 g) milk protein doses comparably impacted EIMD responses. Therefore, this finding highlights the disconnect between EIMD with MPS and hypertrophy processes and supports that recovery from EIMD is not enhanced by sufficient MPS stimulation (Pavis et al., 2021). It follows that at the level of muscle, ingested protein-derived amino acids act differently upon EIMD pathways compared with MPS pathways; however, it is unclear why the EIMD response to protein ingestion is inconsistent.

Inconsistent responses to milk protein ingestion were highlighted by the meta-analysis presented in *chapter 3* that found significant ESs in favour of milk protein relative to control supplementation for the change in MVC (Cockburn et al., 2010; 2012; Draganidis et al., 2017; Rankin et al., 2015), muscle soreness (Cockburn et al., 2008; 2012; Draganidis et al., 2017; Hirose et al., 2013; Rankin et al., 2015) and [CK] (Cockburn et al., 2010; 2012; Rankin et al., 2015) for at least one post-exercise time-point. Nonetheless, in several trials, the milk protein supplement was comparable or inferior to the control for mitigating EIMD symptoms. It is surprising that, in some cases, a single dose of milk protein attenuated EIMD (Cockburn et al., 2010; 2012; Rankin et al., 2015), whereas a total of 17 doses had no positive impact relative to the control (*chapter 4*). The lack of effect was unlikely due to the protein dose, as equivalent (~20 g) and lower protein doses have effectively reduced EIMD (Cockburn et al., 2008; 2012;

Draganidis et al., 2017; Rankin et al., 2015). The population studied was also an unlikely contributor to these outcomes, considering that milk protein has previously shown to attenuate EIMD in untrained males (Cooke et al., 2010; Hirose et al., 2013; Ives et al., 2017; Nieman et al., 2020). Therefore, it is probable that the ecologically-valid exercise protocol utilised, and consequent mild muscle damage, was primarily responsible for the absence of a treatment effect (*chapter 4*) that is otherwise usually present (*chapter 3*). This difference indicated that protein supplementation may only be beneficial for attenuating severe muscle damage or, that in general, treatment effects are easier to identify when the magnitude of EIMD is greater. Given the evidence for the efficacy of protein supplementation for EIMD management is conflicting, other strategies were considered in this thesis.

Alternative strategies for mitigating EIMD have evolved from manipulating resistance exercise variables, such as the contraction type, movement velocity, exercise volume, inter-set rest period, and exercise load. This thesis has showcased a benefit of utilising lower resistance exercise loads for attenuating muscle damage and accelerating post-exercise recovery in young males (*chapter 5*). Resistance training with low exercise loads stimulated comparable muscle hypertrophy and strength gains to training with high loads (Schoenfeld et al., 2017_a). Taken together, these findings have provided further substantiation that severe muscle damage is not a precondition for muscle adaptation, given that low-load resistance exercise is less damaging than high-load exercise, but induces comparable adaptation. Another reason for advocating exercise with lower loads its perception of being less exerting (Arazi & Asadi, 2018; Richardson et al., 2018) resulting in greater exercise compliance than when more exerting, high-load exercise is performed. Therefore, a pragmatic recommendation is to incorporate low-load resistance exercise into training programmes. This exercise prescription particularly might appeal to individuals who are new to resistance training, elderly, or recovering from injury and hesitant to utilise heavy loads. Although, these recommendations currently may apply exclusively to males until supporting research is conducted with females.

As well as those easily-manipulated extrinsic factors, such as nutrition and exercise, various intrinsic factors have been explored in the context of EIMD that include age, sex, hormonal status, training status, and body composition. The influence of sex and body composition on EIMD were focussed on in this thesis, and it was hypothesised that differences in body composition between males and females may partly account for the sex divergencies in EIMD. To this end, significant associations between body composition outcomes and exercise-induced changes in [CK] in untrained females, but not males, were identified in the correlational

analyses conducted in *chapter 4*. Greater post-exercise [CK] elevations in males and females with higher compared with lower BF% have been reported by previous studies (Heled et al., 2007; Kim & Lee, 2015; Margaritelis et al., 2019; Paschalis et al., 2010; 2013). Opposingly, negative relationships in females between post-exercise [CK] increases and several total and regional body composition outcomes, including BF%, were found in this thesis. While the reasons for this discrepancy require further investigation, a potential cause relates to the female participants here (*chapter 4*) being healthy weight, whereas in most earlier studies females were overweight/obese. This suggests that the influence of adipose tissues on myofibre membrane integrity depends on whether BF% is within a healthy range, with higher body fat offering an advantage in non-obese females. Notwithstanding, this thesis has added to the current work as the first study to relate DXA-derived total and regional body composition with EIMD, while most prior studies utilised body composition assessment methods that do not allow for precise regional analysis. Moreover, previous studies were conducted with either one sex or did not test for sex differences. Since significant associations were present in females yet absent in males, the importance of exploring sex differences in the relationship of body composition and EIMD has been highlighted in this thesis.

7.1.2. Sex Differences in EIMD

An overarching theme of sex differences has been held by this thesis, and various physiological differences between adult males and females, not limited to hormone milieu, body composition, skeletal musculature, and acute and chronic responses to exercise, have been discussed. Based on these central differences, it is not surprising that management strategies for EIMD may vary between males and females. However, at present, there have been insufficient high-quality studies conducted with females or both sexes to confirm the influence of sex on EIMD at a rudimentary level. Studies involving females have produced conflicting outcomes, which is likely because most studies – perhaps with the exception of those conducted in recent years – are of poor methodological quality and lack consideration for female-specific study designs e.g. with control for or merely reporting of MC phase, HC use, and menopausal status (Elliott-Sale et al., 2021).

Studies examining sex differences in the response to EIMD management strategies are of even lower number. Regarding the impact of resistance exercise load on EIMD, reviewing of the current literature identified that 97% of research participants were male, with only one study involving male and female older adults (*chapter 5*). To address this sex data gap, a study

protocol for a randomised, parallel-group trial investigating the impact of exercise load on muscle damage and recovery following resistance exercise in young, untrained males and females was described in this thesis (*chapter 6*). Female-specific methodological controls, for example by conducting measurements during a consistent MC phase, analysing serum oestradiol concentration, and accounting for HC use, were considered in this protocol in attempt to establish the influence of sex on EIMD. Likewise, regarding the impact of dietary protein supplementation on resistance EIMD, research to date has been heavily male dominated. Out of the 763 participants involved in the studies included in the systematic review, 94% were young males and only one study involved both sexes (*chapter 3*).

Accordingly, the outcomes of an RCT conducted with young, untrained males and females to examine sex differences in the EIMD response to milk protein ingestion were presented in this thesis (*chapter 4*). As hypothesised, sex differences were apparent. Females experienced attenuated muscle damage following acute resistance exercise relative to males; specifically, peak post-exercise increases in [CK] and reductions in PPT were lower, and females had accelerated recovery from muscle soreness. In this study, all measurements were conducted during the estimated late-follicular phase of the MC when oestrogen concentration was rising, but not peaked. Therefore, these study outcomes indicated that the oestrogenic environment in females offered protection against EIMD, as previously shown (Romero-Parra et al., 2021_a; Tiidus, 2003), compared with males. This effect was perhaps mediated by an oestrogen-induced stabilisation of myofibre membranes (Amelink & Bär, 1986; Amelink et al., 1990; Bär et al., 1988) resulting in a lower [CK] response. Increased myofibre membrane permeability during exercise also might explain the sex differences in PPT. Pain threshold may be lowered due to the histamine-mediated sensitisation of nociceptors that can occur after myofibre membranes are weakened and calcium homeostasis is lost. Thus, it is plausible that males experienced a greater sensitisation of nociceptors and hence lower pain threshold relative to females, although the role of nociceptors in exercise-induced muscle soreness is not yet confirmed. It is unlikely that sex differences in EIMD were driven by sex differences in body composition because, as identified in *chapter 4*, body composition outcomes were only related to peak changes in [CK] and not to other markers of EIMD. Furthermore, possessing greater body mass and LBM was favourable for limiting [CK] elevations in females but not males, despite males having higher body mass and LBM. As such, it is most likely that other factors, notably differences between males and females in oestrogen status, primarily drive sex differences in EIMD.

7.2.0. Directions for Future Research

This thesis has contributed to the existing knowledge on factors influencing EIMD and potential management strategies, although several remaining knowledge gaps were identified. Research priority in this field should be placed on addressing the sex data gap by conducting high-quality studies in both sexes or replicating existing methods in females, with consideration for female-specific study designs in accordance with recent guidance (Elliott-Sale et al., 2021). Once the understanding of how sex impacts central EIMD responses is improved, this knowledge should be used to inform appropriate study designs to investigate management strategies for EIMD in both sexes. The study protocol outlined in *chapter 6* may be conducted as described or used as a foundation for developing studies to examine the impact of extrinsic exercise variables on EIMD in males and females. Furthermore, there are several knowledge gaps relating to the mechanisms of EIMD symptom development and management and optimal markers for EIMD assessment.

The mechanisms of exercise-induced muscle soreness are not well understood, though the outcomes of this thesis suggested that muscle soreness does not have a mechanical aetiology. As found in *Chapter 4*, resistance exercise triggered increases in muscle soreness and reductions in pain threshold in the absence of maximal strength decrements. Muscle force loss is a consequence of mechanical damage (sarcomere overextension), and the fact that strength was not impaired suggests that mechanical damage was either not significant or restored by the first assessment (+72 h). Meanwhile muscle soreness persisted for 72 h post-exercise. This finding implied that muscle soreness perhaps relates to the culminative consequences of increased membrane permeability (i.e. efflux of intramuscular enzymes, local inflammation, disrupted calcium homeostasis, sensitisation of nociceptors). This pathway seems logical considering that [CK] also was significantly elevated, indicative of increased membrane permeability. In this instance, assessing cytosolic [Ca^{2+}] and local inflammation (e.g. by ultrasound) and relating these outcomes to muscle soreness data would have been beneficial to corroborate this theory. Alternatively, muscle soreness has been proposed to relate to altered behaviour of muscle fascia connective tissue (Peñailillo et al., 2015; Wilke & Behringer, 2021). Yet, this thesis cannot develop this notion because muscle fascia properties – for example biomarkers of connective tissue/collagen breakdown – were not assessed. Thus, it cannot be known whether muscle fascia behaviour played a role in soreness symptoms here. Additional

studies are required to build a bigger picture of the factors influencing and strategies to reduce the severity of muscle soreness, which may then allude to its aetiology. Factors to be first explored are connective tissue properties, local inflammation (Philpott et al., 2018) (e.g. by implementing strategies that reduce local inflammation and examining the concurrent impact on muscle soreness), and extracellular matrix composition and functioning (Hyldahl et al., 2015).

Considering several markers of EIMD are subjective and variable, the inclusion of objective markers, such as biomarkers, to corroborate subjective responses is advantageous. However, the use of biomarkers could be improved. Creatine kinase was identified by this thesis as the most frequently assessed biomarker of EIMD (*chapters 3 and 5*) despite its notorious variability (Chrismas et al., 2018; Damas et al., 2016$_a$; Nosaka & Clarkson, 1996$_c$). Inter-individual (*chapter 4*) and inter-study (*chapter 3*) variability in CK responses to exercise with milk protein ingestion were also identified by this thesis. For instance, two similar studies conducted by Cockburn and colleagues utilised the same protein supplementation protocol, exercise protocol, and sample, yet ESs significantly favoured milk protein for [CK] in the former (Cockburn et al., 2012) but not latter (Cockburn et al., 2013) study. Such findings question the validity of [CK] as a marker EIMD. Irrespectively, serum [CK] was the chosen biomarker of EIMD in the present RCT (*chapter 4*) so the outcomes could be compared with other studies, which is likely a common justification. Ideally, future studies should assess additional or alternative biomarkers of EIMD, such as myoglobin, fast-twitch skeletal troponin isoforms, or myosin heavy chain fragments (Chen et al., 2020; Sorichter et al., 1997) and gradually move away from CK as the biomarker of popularity. This could be achieved by researchers assessing [CK] plus an additional marker, and once there are sufficient data on the additional markers, researchers could stop measuring [CK].

Methodological inconsistencies across EIMD studies were recurringly identified in this thesis and are problematic when attempting to compare between studies, explain contrasting findings, and reach consensuses. For instance, among the studies systematically reviewed (*chapter 3*), 6 different assessment methods were used to measure active muscle soreness across 13 studies. It would therefore be advantageous – particularly for systematic reviews, meta-analyses, and consensus statements – if future studies adopted comparable methods such that, where feasible, dependent variables, except for CK, were alike to previous studies. Specifically, by assessing the same EIMD markers using the same measurement tools at the same time-points in relation to exercise – at least for commonly assessed markers (e.g. MVC and muscle soreness) with any

additional markers being a bonus. Such uniformity would foster confidence in the effectiveness of individual EIMD management strategies.

The efficacy of dietary protein as an EIMD management strategy was investigated in this thesis. Nonetheless, the mechanisms by which dietary protein may act upon EIMD pathways are unknown, and improving understanding of the potential mechanisms is challenged by the inconsistent EIMD responses to ingested protein. Although peri-exercise protein consumption is renowned for its stimulatory effect on MPS rates, an involvement of MPS in EIMD is doubtful. The time course for damaged muscle proteins to be remodelled and regain functionality does not align with the acute nature of EIMD and its recovery. However, EIMD might relate to MPB. Rates of MPB are elevated following resistance exercise to increase the availability of free amino acids (Biolo et al., 1995), and it is possible that membrane structural proteins are among those broken down; thus, weakening membrane integrity. An increased exogenous supply of amino acids reduces MPB rates (Tipton et al., 2018), and therefore, peri-exercise protein ingestion may help preserve myofibre membrane integrity by limiting MPB. However, investigating the connection between protein supplementation, MPB, and EIMD is challenging because the direct assessment of MPB rates can only be completed at the level of mixed muscle and not subcellular fractions (e.g. myofibrillar, sarcoplasmic). Furthermore, the impact of the amino acid composition and quality of the ingested protein on EIMD is poorly understood. Only two studies that compared EIMD responses to ingested plant versus animal protein sources following resistance exercise were identified in *Chapter 3*. While these studies did not report an effect of the quality of ingested protein on EIMD, further corroborative studies, particularly with females, are needed. Likewise, there is limited, if any, research on the influence of food matrices on EIMD, which could be addressed by examining the impact of ingested protein from whole-food or mixed meal compared with isolated sources.

7.3.0. Thesis Limitations

This thesis held some limitations that should be considered when interpreting its outcomes and practical applications. Regarding the RCT (*chapter 4*), uneven sample sizes between males and females were obtained, which may have reduced statistical power and increased type I error risk (Rusticus & Lovato, 2014). Furthermore, the required group sample size (n = 5) was not achieved for Male-PRO and Male-CON (both n = 4); thus, 80% statistical power to detect between-group differences was only reached when male subgroups or MILK-PRO and CON

were pooled. Given a difference between MILK-PRO and CON was not detected, it seemed acceptable to pool the supplement subgroups to test for overall sex differences.

Supplement allocation in the RCT was single-blinded to limit participant bias. Fifty-nine percent of participants did not identify their treatment condition and so blinding can be considered successful. To this end, this thesis advantages prior similar studies (Cockburn et al., 2010; 2012; Rankin et al., 2015; Wojcik et al., 2001) that did not achieve participant nor researcher blinding and were therefore subject to bias – for example, if the participant believed the ingested protein should lower their muscle soreness. Therefore, the current outcomes provided a more valid indication of how milk protein impacts subjective markers of EIMD. Double-blinding was not feasible due to logistical constraints and because the yoghurt supplements were included in the 24 h dietary recall analysis, so treatment condition had to be known by the lead researcher who conducted these analyses. To counter researcher bias, equal verbal encouragement during exercise was given and measures were conducted in the same fashion for all participants.

There were several limitations pertaining to the methods used to assess EIMD – some of which were unavoidable (e.g. due to resource and financial constraints) and others were realised in hindsight after further research or practice. First, the resistance exercise machine used both to assess maximal strength and induce muscle damage had a maximum load capacity of 106.3 kg. Six participants reached this load, which may not have been their true 1RM, and subsequently, the exercise load for the muscle damage protocol (80% 1RM) and any changes in maximal strength could have been inaccurate. Moreover, load increments were limited to 2.3 kg so, typically, the exercise load for the muscle damage protocol was rounded to the nearest increment and not exactly equivalent to 80% 1RM. Ideally, were the facilities available, an isokinetic dynamometer would have been used to assess maximal strength on a continuous load range.

To accurately assess knee joint ROM, measurements should be conducted on a flat surface. However, assessments were conducted on a phlebotomy bed, adjusted into a supine position, meaning it was difficult to achieve a 180° angle or merely a consistent angle for each measurement. Consequently, participants' knee joint starting angle was inconsistent, which likely impacted the ROM. In hindsight, the foot position of the bed should have been adjusted such that knee joint starting angle was consistent for each participant and time-point.

A 10-point VRS was used to assess subjective muscle soreness, because this scale is considered reliable and valid (Alghadir et al., 2018) and has been implemented in previous EIMD studies (Burnley et al., 2010; Nosaka et al., 2006). However, this scale is limited by being restricted to set, whole numbers and participants would often seek an intermediate (i.e. by rating 'a four or a five'); thus, soreness ratings may be over or underestimated. Alternatively, had a 100 mm VAS been used (an unmarked horizontal line with '0 – not sore at all' and '100 – extremely sore' at either end) this would have allowed for intermediate values. Further, VAS removes the psychological element of knowing exactly what rating is being given and has been given previously, as the participant places a mark along the line and its distance from zero is measured by the researcher to determine the numerical value. With VRS, participants may remember what score (0–10) they gave at a previous time-point, which may bias their soreness rating. Furthermore, participants tended to verbally report feeling greater muscle soreness when walking – particularly on a gradient or stairs – compared with when performing the single bodyweight squat, and so, alternative or additional measurements of active soreness would have been beneficial and perhaps more reflective of their true soreness. Although, this was not known until the data collection process had begun. Muscle soreness was assessed for four muscle groups to account for the fact soreness may vary between muscle groups, and the muscle group experiencing peak soreness may differ between participants.

To mirror prior studies, serum samples were analysed for [CK] (*chapter 4*). However, owing to the inter- and intra- individual variability of [CK], it would have been advantageous to analyse other serum markers of muscle damage, such as myoglobin, skeletal troponins, or myosin heavy chain fragments, to corroborate the CK response, as well as markers of inflammation, such as IL-6, to corroborate muscle swelling data. Regarding the CK data obtained, there was an unknown issue with two of the assay tests, such that absorbance values were greatly beneath the expected range, which invalidated data from five participants. There was an insufficient number of assay kits to repeat all the affected samples, and so, the decision was made to re-analyse three key time-points for each sample: pre, +48 and +72 h to capture baseline and peak concentrations. As such, sample sizes were uneven across time-points.

Regarding the systematic review and meta-analysis (*chapter 3*), a potential limitation was that the study inclusion criteria were restricted to studies that examined EIMD responses to protein supplementation alone and did not include studies involving amino acid supplementation. These inclusion criteria were specified as they were relevant to the wider aims of this thesis. However, investigating both protein and amino acid supplementation may have provided useful

sub-analyses and alluded as to whether the amino acid composition of proteins influences their impact on EIMD. For instance, BCAA supplementation has shown to reduce post-exercise [CK] elevations (Rahimi et al., 2017), and therefore, protein sources with a high BCAA content may attenuate [CK] relative to protein sources with a low BCAA content.

7.4.0. Practical Applications

Two key practical applications have been produced by this thesis. Dietary protein should be consumed peri-exercise to enhance several domains of post-exercise recovery, including recovery from resistance EIMD, as identified in *chapter 3*. However, protein supplementation is not beneficial for attenuating muscle soreness following resistance exercise, and so, alternative strategies for managing soreness symptoms should be investigated. Furthermore, the optimal type, timing, and dose of ingested protein for the management of EIMD remains unknown; although, sub-analyses conducted in *chapter 3* indicated that these factors were not greatly important in the context of EIMD. Hence for now, it is pragmatic for exercising individuals to follow the current protein recommendations for exercise recovery i.e. 20–40 g of high-quality protein ingested peri-exercise. Notwithstanding, under conditions of mild muscle damage, this protein feeding strategy is seemingly insufficient for promoting recovery from resistance EIMD (*chapter 4*), and so, additional strategies for the management of mild EIMD ought to be investigated and implemented.

Resistance training programmes for young males should incorporate low-load exercise, such that exercise loads equivalent to ≤70% 1RM are often or exclusively used to reduce muscle damage and accelerate muscle recovery. Progressive overload can be achieved while practicing low-load resistance training by incrementally increasing the exercise load up to 70% 1RM, and thereafter, the number of performed repetitions and sets can be increased. It is unlikely in females that low-load resistance training would be detrimental in terms of EIMD, although until sex-specific supporting research is completed (e.g. as per the *chapter 6* protocol), practical application of these thesis outcomes may apply exclusively to males.

7.5.0. Overall Conclusion

Overall, it was identified in this thesis that responses to EIMD mostly differed between males and females, yet there is a distinct lack of sex-comparative and female-focussed research in

this area. Recovery from mild muscle damage induced by habitual-type resistance exercise was not enhanced by milk protein supplementation in untrained males and females, and thus, protein supplementation may only be an effective management strategy for more severe EIMD. Alternatively, EIMD in males can be attenuated by utilising lower exercise loads, although supporting evidence in females is needed.

REFERENCES

Aagaard, P., Simonsen, E. B., Trolle, M., et al. (1996). Specificity of training velocity and training load on gains in isokinetic knee joint strength. *Acta Physiologica Scandinavica, 156*(2), 123-129. doi:https://doi.org/10.1046/j.1365-201X.1996.438162000.x

Abe, T., Wong, V., Dankel, S. J., et al. (2020). Skeletal muscle mass in female athletes: The average and the extremes. *American Journal of Human Biology, 32*(2), e23333. doi:https://doi.org/10.1002/ajhb.23333

Abreu, P., Mendes, S. V. D., Ceccatto, V. M., et al. (2017). Satellite cell activation induced by aerobic muscle adaptation in response to endurance exercise in humans and rodents. *Life Sciences, 170*, 33-40. doi:https://doi.org/10.1016/j.lfs.2016.11.016

ACSM. (2009). Progression models in resistance training for healthy adults. *Medicine & Science in Sports & Exercise, 41*(3), 687-708. doi:10.1249/MSS.0b013e3181915670

Al-Azzawi, F. & Palacios, S. (2009). Hormonal changes during menopause. *Maturitas, 63*(2), 135-137. doi:https://doi.org/10.1016/j.maturitas.2009.03.009

Alghadir, A. H., Anwer, S., Iqbal, A., et al. (2018). Test-retest reliability, validity, and minimum detectable change of visual analog, numerical rating, and verbal rating scales for measurement of osteoarthritic knee pain. *Journal of pain research, 11*, 851-856. doi:10.2147/JPR.S158847

Allen, D. (2001). Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiologica Scandinavica, 171*(3), 311-319.

Allen, D., Lannergren, J. & Westerblad, H. (1995). Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Experimental Physiology: Translation and Integration, 80*(4), 497-527.

Alvarez, I. F., Damas, F., Biazon, T. M. P. D., et al. (2020). Muscle damage responses to resistance exercise performed with high-load versus low-load associated with partial blood flow restriction in young women. *European Journal of Sport Science, 20*(1), 125-134. doi:10.1080/17461391.2019.1614680

Amelink, G. J. & Bär, P. R. (1986). Exercise-induced muscle protein leakage in the rat: Effects of hormonal manipulation. *Journal of the neurological sciences, 76*(1), 61-68. doi:https://doi.org/10.1016/0022-510X(86)90142-5

Amelink, G. J., Koot, R. W., Erich, W. B. M., et al. (1990). Sex-linked variation in creatine kinase release, and its dependence on oestradiol, can be demonstrated in an in-vitro rat

skeletal muscle preparation. *Acta Physiologica Scandinavica, 138*(2), 115-124. doi:https://doi.org/10.1111/j.1748-1716.1990.tb08823.x

Amelink, G. J., Van der Kallen, C. J. H., Wokke, J. H. J., et al. (1990). Dantrolene sodium diminishes exercise-induced muscle damage in the rat. *European Journal of Pharmacology, 179*(1), 187-192. doi:https://doi.org/10.1016/0014-2999(90)90417-5

Andersen, J. L., Schjerling, P. & Saltin, B. (2000). Muscle, genes and athletic performance. *Scientific American, 283*(3), 48-55.

Anderson, K & Behm, D. G. (2005). The impact of instability resistance training on balance and stability. *Sports Medicine, 35*(1), 43-53. doi:10.2165/00007256-200535010-00004

Anderson, T & Kearney, J. T. (1982). Effects of three resistance training programs on muscular strength and absolute and relative endurance. *Research Quarterly for Exercise and Sport, 53*(1), 1-7.

Andersson, A., Nälsén, C., Tengblad, S., et al. (2002). Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. *Am J Clin Nutr, 76*(6), 1222-1229. doi:10.1093/ajcn/76.6.1222

Andersson, H., Karlsen, A., Blomhoff, R., et al. (2010). Plasma antioxidant responses and oxidative stress following a soccer game in elite female players. *Scandinavian Journal of Medicine & Science in Sports, 20*(4), 600-608. doi:https://doi.org/10.1111/j.1600-0838.2009.00987.x

Ansdell, P., Thomas, K., Hicks, K. M., et al. (2020). Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Experimental Physiology, 105*(12), 2007-2021. doi:https://doi.org/10.1113/EP088548

Aragón-Vela, J., Fontana, L., Casuso, R. A., et al. (2021). Differential inflammatory response of men and women subjected to an acute resistance exercise. *Biomedical journal, 44*(3), 338-345. doi:10.1016/j.bj.2020.02.005

Arazi, H. & Asadi, A. (2013). One repetition maximum test increases serum indices of muscle damage and soreness in trained and untrained males. *Apunts. Medicina de l'Esport, 48*(178), 49-54.

Arazi, H. & Asadi, A. (2018). Effects of leg press exercise intensity on symptoms of muscle damage and soreness in men. *Med. Sport (Roma), 71*(2), 174-189.

Areta, J. L., Burke, L. M., Ross, M. L., et al. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of Physiology, 591*(9), 2319-2331. doi:https://doi.org/10.1113/jphysiol.2012.244897

Armstrong, R. B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and science in sports and exercise, 16*(6), 529-538.

Armstrong, R. B., Ogilvie, R. & Schwane, J. (1983). Eccentric exercise-induced injury to rat skeletal muscle. *Journal of Applied Physiology, 54*(1), 80-93.

Armstrong, R. B., Warren, G. L. & Warren, J. A. (1991). Mechanisms of exercise-induced muscle fibre injury. *Sports Med, 12*(3), 184-207. doi:10.2165/00007256-199112030-00004

Arnal, M. A., Mosoni, L., Boirie, Y., et al. (1999). Protein pulse feeding improves protein retention in elderly women. *The American journal of clinical nutrition, 69*(6), 1202-1208. doi:10.1093/ajcn/69.6.1202

Arnal, M. A., Mosoni, L., Boirie, Y., et al. (2000). Protein feeding pattern does not affect protein retention in young women. *The Journal of nutrition, 130*(7), 1700-1704. doi:10.1093/jn/130.7.1700

Arnold, L., Henry, A., Poron, F., et al. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *The Journal of experimental medicine, 204*(5), 1057-1069.

Asadi, A., Arazi, H. & Suzuki, K. (2017). Effects of β-hydroxy-β-methylbutyrate-free acid supplementation on strength, power and hormonal adaptations following resistance training. *Nutrients, 9*(12). doi:10.3390/nu9121316

Ashton, T., Rowlands, C. C., Jones, E., et al. (1998). Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. *Eur J Appl Physiol Occup Physiol, 77*(6), 498-502. doi:10.1007/s004210050366

Atherton, P. J., Kumar, V., Selby, A. L., et al. (2017). Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clinical nutrition, 36*(3), 888-895. doi:https://doi.org/10.1016/j.clnu.2016.04.025

Au, J. S., Oikawa, S. Y., Morton, R. W., et al. (2017). Arterial stiffness is reduced regardless of resistance training load in young men. *Medicine & Science in Sports & Exercise, 49*(2), 342-348.

Babault, N., Pousson, M., Ballay, Y., et al. (2001). Activation of human quadriceps femoris during isometric, concentric, and eccentric contractions. *Journal of Applied Physiology, 91*(6), 2628-2634. doi:10.1152/jappl.2001.91.6.2628

Baechle, T. R. & Earle, R. W. (2008). *Essentials of strength training and conditioning*: Human kinetics.

Bailey, D. M., Lawrenson, L., McEneny, J., et al. (2007). Electron paramagnetic spectroscopic evidence of exercise-induced free radical accumulation in human skeletal muscle. *Free Radical Research, 41*(2), 182-190. doi:10.1080/10715760601028867

Baird, M. F., Graham, S. M., Baker, J. S., et al. (2012). Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *Journal of Nutrition and Metabolism, 2012*, 960363. doi:10.1155/2012/960363

Baltusnikas, J., Venckunas, T., Kilikevicius, A., et al. (2015). Efflux of creatine kinase from isolated soleus muscle depends on age, sex and type of exercise in mice. *Journal of sports science & medicine, 14*(2), 379-385. Retrieved from https://pubmed.ncbi.nlm.nih.gov/25983588

Bär, P. R., Amelink, G. J., Oldenburg, B., et al. (1988). Prevention of exercise-induced muscle membrane damage by oestradiol. *Life Sciences, 42*(26), 2677-2681. doi:https://doi.org/10.1016/0024-3205(88)90243-3

Baracos, V. E. (1984). Calcium ions and the regulation of intracellular protein breakdown in muscle. *Calcium regulation in biological systems*.

Barcelos, L. C., Nunes, P. R. P., de Souza, L. R. M. F., et al. (2015). Low-load resistance training promotes muscular adaptation regardless of vascular occlusion, load, or volume. *European journal of applied physiology, 115*(7), 1559-1568. doi:10.1007/s00421-015-3141-9

Barroso, R., Roschel, H., Gil, S., et al. (2011). Effect of number and intensity of eccentric actions on muscle damage indicators. *Rev Bras Med Esporte, 17*(6), 401-404. https://doi.org/10.1590/S1517-86922011000600006

Bartolomei, S., Sadres, E., Church, D. D., et al. (2017). Comparison of the recovery response from high-intensity and high-volume resistance exercise in trained men. *European journal of applied physiology, 117*(7), 1287-1298. doi:10.1007/s00421-017-3598-9

Baty, J. J., Hwang, H., Ding, Z., et al. (2007). The effect of a carbohydrate and protein supplement on resistance exercise performance, hormonal response, and muscle damage. *J Strength Cond Res, 21*(2), 321-329. doi:10.1519/r-21706.1

Beaton, L. J., Tarnopolsky, M. A. & Phillips, S. M. (2002). Contraction-induced muscle damage in humans following calcium channel blocker administration. *The Journal of Physiology, 544*(3), 849-859. doi:https://doi.org/10.1113/jphysiol.2002.022350

Beck, T. W., Kasishke, P. R. I., Stock, M. S., et al. (2012). Neural contributions to concentric vs. eccentric exercise–induced strength loss. *The Journal of Strength & Conditioning Research, 26*(3), 633-640. doi:10.1519/JSC.0b013e3182474296

Beckwée, D., Delaere, A., Aelbrecht, S., et al. (2019). Exercise interventions for the prevention and treatment of sarcopenia. A systematic umbrella review. *The journal of nutrition, health & aging, 23*(6), 494-502. doi:10.1007/s12603-019-1196-8

Beelen, M., Tieland, M., Gijsen, A. P., et al. (2008). Coingestion of carbohydrate and protein hydrolysate stimulates muscle protein synthesis during exercise in young men, with no further increase during subsequent overnight recovery. *The Journal of nutrition, 138*(11), 2198-2204. doi:10.3945/jn.108.092924

Bekkelund, S. I. & Jorde, R. (2018). Creatine kinase in relation to body fat in a Caucasian overweight and obese population. *Scandinavian Journal of Clinical and Laboratory Investigation, 78*(1-2), 43-48. doi:10.1080/00365513.2017.1408140

Benini, R., Prado Nunes, P. R., Orsatti, C. L., et al. (2015). Effects of acute total body resistance exercise on hormonal and cytokines changes in men and women. *J Sports Med Phys Fitness, 55*(4), 337-344.

Biolo, G., Maggi, S. P., Williams, B. D., et al. (1995). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *American Journal of Physiology-Endocrinology and Metabolism, 268*(3), E514-E520. doi:10.1152/ajpendo.1995.268.3.E514

Bird, S. P., Mabon, T., Pryde, M., et al. (2013). Triphasic multinutrient supplementation during acute resistance exercise improves session volume load and reduces muscle damage in strength-trained athletes. *Nutrition Research, 33*(5), 376-387. doi:10.1016/j.nutres.2013.03.002

Bisdee, J. T., James, W. P. T. & Shaw, M. A. (1989). Changes in energy expenditure during the menstrual cycle. *British Journal of Nutrition, 61*(2), 187-199. doi:10.1079/BJN19890108

Bishop, P., Cureton, K. & Collins, M. (1987). Sex difference in muscular strength in equally-trained men and women. *Ergonomics, 30*(4), 675-687.

Bloomer, R. J., Goldfarb, A., Wideman, L., et al. (2005). Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *Journal of strength and conditioning research, 19*, 276-285. doi:10.1519/14823.1

Bloomer, R. J., Goldfarb, A. H. & McKenzie, M. J. (2006). Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. *Medicine and science in sports and exercise, 38*(6), 1098-1105. doi:10.1249/01.mss.0000222839.51144.3e

Bolgla, L., Cook, N., Hogarth, K., et al. (2014). Trunk and hip electromyographic activity during single leg squat exercises do sex differences exist? *International journal of*

sports physical therapy, 9(6), 756-764. Retrieved from https://pubmed.ncbi.nlm.nih.gov/25383244

Bondarev, D., Laakkonen, E. K., Finni, T., et al. (2018). Physical performance in relation to menopause status and physical activity. *Menopause, 25*(12), 1432-1441. doi:10.1097/gme.0000000000001137

Bonetto, A. & Bonewald, L. F. (2019). Bone and muscle. In *Basic and Applied Bone Biology* (pp. 317-332): Elsevier.

Borack, M. S., Reidy, P. T., Husaini, S. H., et al. (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. *The Journal of nutrition, 146*(12), 2468-2475. doi:10.3945/jn.116.231159

Borenstein, M., Hedges, L. V., Higgins, J. P., et al. (2021). *Introduction to meta-analysis*: John Wiley & Sons.

Boss, L., Kang, D-H., Marcus, M., et al. (2014). Endogenous sex hormones and cognitive function in older adults: a systematic review. *Western journal of nursing research, 36*(3), 388-426.

Breen, L. & Phillips, S. M. (2011). Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing. *Nutrition & metabolism, 8*(1), 68. doi:10.1186/1743-7075-8-68

Bridge, A., Brown, J., Snider, H., et al. (2019). Greek yogurt and 12 weeks of exercise training on strength, muscle thickness and body composition in lean, untrained, university-aged males. *Frontiers in Nutrition, 6*, 55-55. doi:10.3389/fnut.2019.00055

Brockett, C. L., Morgan, D. L. & Proske, U. (2001). Human hamstring muscles adapt to eccentric exercise by changing optimum length. *Medicine and science in sports and exercise, 33*(5), 783-790. doi:10.1097/00005768-200105000-00017

Brooke, M. H. & Engel, W. K. (1969). The histographic analysis of human muscle biopsies with regard to fiber types. *I. Adult male and female, 19*(3), 221-221. doi:10.1212/wnl.19.3.221

Brooks, S. V., Zerba, E. & Faulkner, J. A. (1995). Injury to muscle fibres after single stretches of passive and maximally stimulated muscles in mice. *J Physiol, 488 (Pt 2)*(Pt 2), 459-469. doi:10.1113/jphysiol.1995.sp020980

Brose, A., Parise, G. & Tarnopolsky, M. A. (2003). Creatine supplementation enhances isometric strength and body composition improvements following strength exercise

training in older adults. *The Journals of Gerontology: Series A, 58*(1), B11-B19. doi:10.1093/gerona/58.1.B11

Brown, S. J., Child, R. B., Day, S. H., et al. (1997). Indices of skeletal muscle damage and connective tissue breakdown following eccentric muscle contractions. *European Journal of Applied Physiology and Occupational Physiology, 75*(4), 369-374. doi:10.1007/s004210050174

Brown, M. A., Howatson, G., Keane, K., et al. (2015). Exercise-induced muscle damage following dance and sprint specific exercise in females. *J. Sports Med. Phys. Fit, 56*, 1376-1383.

Brown, M. A., Stevenson, E. J. & Howatson, G. (2018). Whey protein hydrolysate supplementation accelerates recovery from exercise-induced muscle damage in females. *Appl Physiol Nutr Metab, 43*(4), 324-330. doi:10.1139/apnm-2017-0412

Brown, M. A., Stevenson, E. J. & Howatson, G. (2019). Montmorency tart cherry (Prunus cerasus L.) supplementation accelerates recovery from exercise-induced muscle damage in females. *Eur J Sport Sci, 19*(1), 95-102. doi:10.1080/17461391.2018.1502360

Bruce, C. D., Ruggiero, L., Dix, G. U., et al. (2021). Females and males do not differ for fatigability, muscle damage and magnitude of the repeated bout effect following maximal eccentric contractions. *Appl Physiol Nutr Metab, 46*(3), 238-246. doi:10.1139/apnm-2020-0516

Brusco, C. M., Blazevich, A. J., Radaelli, R., et al. (2018). The effects of flexibility training on exercise-induced muscle damage in young men with limited hamstrings flexibility. *Scandinavian Journal of Medicine & Science in Sports, 28*(6), 1671-1680.

Buckley, J. D., Thomson, R. L., Coates, A. M., et al. (2010). Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *Journal of Science & Medicine in Sport, 13*(1), 178-181.

Buckley, J. D., Thomson, R. L., Coates, A. M., et al. (2010). Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *J Sci Med Sport, 13*(1), 178-181. doi:10.1016/j.jsams.2008.06.007

Bukhari, S. S. I., Phillips, B. E., Wilkinson, D. J., et al. (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. *American Journal of Physiology-Endocrinology and Metabolism, 308*(12), E1056-E1065. doi:10.1152/ajpendo.00481.2014

Bull, F. C., Al-Ansari, S. S., Biddle, S., et al. (2020). World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *British journal of sports medicine, 54*(24), 1451-1462. doi:10.1136/bjsports-2020-102955

Burd, N. A., Holwerda, A. M., Selby, K. C., et al. (2010). Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *The Journal of Physiology, 588*(16), 3119-3130.

Burd, N. A., Beals, J. W., Martinez, I. G., et al. (2019). Food-first approach to enhance the regulation of post-exercise skeletal muscle protein synthesis and remodeling. *Sports Medicine, 49*(1), 59-68.

Burd, N. A., West, D. W., Staples, A. W., et al. (2010). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS One, 5*(8), e12033. doi:10.1371/journal.pone.0012033

Burd, N. A., Yang, Y., Moore, D. R., et al. (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*(6), 958-962.

Burnley, E. C. D., Olson, A. N., Sharp, R. L., et al. (2010). Impact of protein supplements on muscle recovery after exercise-induced muscle soreness. *Journal of Exercise Science and Fitness, 8*(2), 89-96. doi:10.1016/S1728-869X(10)60014-7

Burzyn, D., Kuswanto, W., Kolodin, D., et al. (2013). A special population of regulatory T cells potentiates muscle repair. *Cell, 155*(6), 1282-1295.

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., et al. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience, 14*(5), 365-376. doi:10.1038/nrn3475

Byrd, S., Bode, A. & Klug, G. A. (1989_a). Effects of exercise of varying duration on sarcoplasmic reticulum function. *Journal of Applied Physiology, 66*(3), 1383-1389.

Byrd, S., McCutcheon, L., Hodgson, D., et al. (1989_b). Altered sarcoplasmic reticulum function after high-intensity exercise. *Journal of Applied Physiology, 67*(5), 2072-2077.

Byrne, C. & Eston, R. (2002). Maximal-intensity isometric and dynamic exercise performance after eccentric muscle actions. *Journal of Sports Sciences, 20*(12), 951-959. doi:10.1080/026404102321011706

Cable, N. T. & Elliott, K. J. (2004). The influence of reproductive hormones on muscle strength. *Biological Rhythm Research, 35*(3), 235-244. doi:10.1080/09291010412331335788

Cadore, E. L., González-Izal, M., Grazioli, R., et al. (2018). Effects of concentric and eccentric strength training on fatigue induced by concentric and eccentric exercise. *Int J Sports Physiol Perform*, 1-30. doi:10.1123/ijspp.2018-0254

Cagnacci, A., De Toni, A., Caretto, S., et al. (2006). Cyclic progestin administration increases energy expenditure and decreases body fat mass in perimenopausal women. *Menopause, 13*(2), 197-201. doi:10.1097/01.gme.0000174469.37198.a0

Campos, G. E., Luecke, T. J., Wendeln, H. K., et al. (2002). Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *European journal of applied physiology, 88*(1-2), 50-60.

Carafoli, E. (1985). The homeostasis of calcium in heart cells. *Journal of molecular and cellular cardiology, 17*(3), 203-212.

Carlsson, T., Wedholm, L., Nilsson, J., et al. (2017). The effects of strength training versus ski-ergometer training on double-poling capacity of elite junior cross-country skiers. *European journal of applied physiology, 117*(8), 1523-1532. doi:10.1007/s00421-017-3621-1

Carpenter, S. & Karpati, G. (1989). Segmental necrosis and its demarcation in experimental micropuncture injury of skeletal muscle fibers. *Journal of Neuropathology & Experimental Neurology, 48*(2), 154-170.

Carter, A., Dobridge, J. & Hackney, A. (2001). Influence of estrogen on markers of muscle tissue damage following eccentric exercise. *Human Physiology, 27*(5), 626-630.

Casale, R. (1988). Acute low back pain: symptomatic treatment with a muscle relaxing drug. *Knee, 6*, 5.

Casanova, N., Reis, J. F., Vaz, J. R., et al. (2018). Effects of roller massager on muscle recovery after exercise-induced muscle damage. *Journal of Sports Sciences, 36*(1), 56-63. doi:10.1080/02640414.2017.1280609

Castell, G. S., Serra-Majem, L. & Ribas-Barba, L. (2015). What and how much do we eat? 24-hour dietary recall method. *Nutricion hospitalaria, 31*(3), 46-48.

Castiglioni, A., Corna, G., Rigamonti, E., et al. (2015). FOXP3+ T cells recruited to sites of sterile skeletal muscle injury regulate the fate of satellite cells and guide effective tissue regeneration. *PLoS One, 10*(6), e0128094.

Cermak, N. M., Noseworthy, M. D., Bourgeois, J. M., et al. (2012). Diffusion tensor MRI to assess skeletal muscle disruption following eccentric exercise. *Muscle & Nerve, 46*(1), 42-50. doi:https://doi.org/10.1002/mus.23276

Chapman, D. W., Newton, M., McGuigan, M., et al. (2008). Effect of lengthening contraction velocity on muscle damage of the elbow flexors. *Medicine and science in sports and exercise, 40*(5), 926-933. doi:10.1249/MSS.0b013e318168c82d

Charkoudian, N. & Stachenfeld, N. (2016). Sex hormone effects on autonomic mechanisms of thermoregulation in humans. *Autonomic Neuroscience, 196*, 75-80. doi:https://doi.org/10.1016/j.autneu.2015.11.004

Chazaud, B. (2016). Inflammation during skeletal muscle regeneration and tissue remodeling: application to exercise-induced muscle damage management. *Immunol Cell Biol, 94*(2), 140-145. doi:10.1038/icb.2015.97

Chazaud, B., Sonnet, C., Lafuste, P., et al. (2003). Satellite cells attract monocytes and use macrophages as a support to escape apoptosis and enhance muscle growth. *Journal of Cell Biology, 163*(5), 1133-1143.

Chen, T. C. (2006). Variability in muscle damage after eccentric exercise and the repeated bout effect. *Research Quarterly for Exercise and Sport, 77*(3), 362-371. doi:10.1080/02701367.2006.10599370

Chen, T. C., Chen, H-L., Lin, M-J., et al. (2016). Contralateral repeated bout effect of eccentric exercise of the elbow flexors. *Medicine and science in sports and exercise, 48*(10), 2030-2039. doi:10.1249/mss.0000000000000991

Chen, T. C., Chen, H-L., Pearce, A. J., et al. (2012). Attenuation of eccentric exercise–induced muscle damage by preconditioning exercises. *Medicine & Science in Sports & Exercise, 44*(11), 2090-2098.

Chen, T. C., Chen, Y-C., Tung, K., et al. (2019). Effects of caffeine and sex on muscle performance and delayed-onset muscle soreness after exercise-induced muscle damage: a double-blind randomized trial. *Journal of Applied Physiology, 127*(3), 798-805. doi:10.1152/japplphysiol.01108.2018

Chen, T. C., Huang, G. L., Hsieh, C. C., et al. (2020). Comparison among three different intensities of eccentric contractions of the elbow flexors resulting in the same strength loss at one day post-exercise for changes in indirect muscle damage markers. *Eur J Appl Physiol, 120*(1), 267-279. doi:10.1007/s00421-019-04272-w

Chen, T. C., Lin, K-Y., Chen, H-L., et al. (2011). Comparison in eccentric exercise-induced muscle damage among four limb muscles. *European journal of applied physiology, 111*(2), 211-223. doi:10.1007/s00421-010-1648-7

Chen, T. C., Nosaka, K., Chen, H-L., et al. (2011). Effects of flexibility training on eccentric exercise-induced muscle damage. *Medicine & Science in Sports & Exercise, 43*(3), 491-500.

Chen, T. C., Nosaka, K. & Sacco, P. (2007). Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. *Journal of Applied Physiology, 102*(3), 992-999. doi:10.1152/japplphysiol.00425.2006

Chen, T. C., Liu, H-W., Russell, A., et al. (2020). Large increases in plasma fast skeletal muscle troponin I after whole-body eccentric exercises. *Journal of Science and Medicine in Sport, 23*(8), 776-781. doi:https://doi.org/10.1016/j.jsams.2020.01.011

Cholewa, J. M., Rossi, F. E., MacDonald, C., et al. (2018). The effects of moderate- versus high-load resistance training on muscle growth, body composition, and performance in collegiate women. *The Journal of Strength & Conditioning Research, 32*(6), 1511-1524. doi:10.1519/jsc.0000000000002048

Chrismas, B., Taylor, L., Smith, A., et al. (2018). Reproducibility of measurement techniques used for creatine kinase, interleukin-6 and high-sensitivity C-reactive protein determination over a 48 h period in males and females. *Measurement in Physical Education and Exercise Science, 22*(3), 191-199.

Churchward-Venne, T. A., Pinckaers, P. J. M., Smeets, J. S. J., et al.(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. *Journal of Nutrition, 149*(2), 210-220.

Clarkson, P. M. & Ebbeling, C. (1988). Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clinical science, 75*(3), 257-261.

Clarkson, P. M. & Hubal, M. J. (2002). Exercise-induced muscle damage in humans. *American Journal of Physical Medicine & Rehabilitation, 81*(11), S52-S69.

Clarkson, P. M., Nosaka, K. & Braun, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and science in sports and exercise, 24*(5), 512-520.

Clarkson, P. M. & Sayers, S. P. (1999). Gender differences in exercise-induced muscle damage. *Gender differences in metabolism: Practical and nutritional implications*, 283-299.

Clifford, T., Ventress, M., Allerton, D. M., et al. (2019). The effects of collagen peptides on muscle damage, inflammation and bone turnover following exercise: a randomized, controlled trial. *Amino Acids, 51*(4), 691-704. doi:10.1007/s00726-019-02706-5

Close, G. L., Ashton, T., Cable, T., et al. (2004). Eccentric exercise, isokinetic muscle torque and delayed onset muscle soreness: the role of reactive oxygen species. *European journal of applied physiology, 91*(5), 615-621. doi:10.1007/s00421-003-1012-2

Close, G. L., Ashton, T., Cable, T., et al. (2005). Effects of dietary carbohydrate on delayed onset muscle soreness and reactive oxygen species after contraction induced muscle damage. *British journal of sports medicine, 39*(12), 948-953. doi:10.1136/bjsm.2005.019844

Close, G. L., Kasper, A., Walsh, N., et al. (2022). "Food First but Not Always Food Only": Recommendations for Using Dietary Supplements in Sport. *International journal of sport nutrition and exercise metabolism*. doi:10.1123/ijsnem.2021-0335

Cockburn, E., Bell, P. G. & Stevenson, E. (2013). Effect of milk on team sport performance after exercise-induced muscle damage. *Medicine and science in sports and exercise, 45*(8), 1585-1592. doi:10.1249/MSS.0b013e31828b7dd0

Cockburn, E., Hayes, P. R., French, D. N., et al. (2008). Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Applied Physiology, Nutrition & Metabolism, 33*(4), 775-783.

Cockburn, E., Robson-Ansley, P., Hayes, P., et al. (2012). Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage. *European journal of applied physiology, 112*(9), 3187-3194.

Cockburn, E., Stevenson, E., Hayes, P. R., et al. (2010). Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage. *Applied Physiology, Nutrition, and Metabolism, 35*(3), 270-277. doi:10.1139/h10-017 %m 20555370

Cockburn, E., Hayes, P. R., French, D. N., et al. (2008). Acute milk-based protein–CHO supplementation attenuates exercise-induced muscle damage. *Applied Physiology, Nutrition, and Metabolism, 33*(4), 775-783. doi:10.1139/h08-057 %m 18641722

Cockburn, E., Robson-Ansley, P., Hayes, P. R., et al. (2012). Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage. *European journal of applied physiology, 112*(9), 3187-3194. doi:10.1007/s00421-011-2288-2

Cohen, J. (1992). A power primer. *Psychological bulletin, 112*(1), 155.

Cohen, J., Manion, L. & Morrison, K. (2007). Research methods in education (Sixth). *Oxon: Routledge*.

Collins, B. C., Arpke, R. W., Larson, A. A., et al. (2019). Estrogen regulates the satellite cell compartment in females. *Cell Reports, 28*(2), 368-381.e366. doi:https://doi.org/10.1016/j.celrep.2019.06.025

Comstock, B. A., Thomas, G. A., Dunn-Lewis, C., et al. (2013). Effects of acute resistance exercise on muscle damage and perceptual measures between men who are lean and obese. *The Journal of Strength & Conditioning Research, 27*(12), 3488-3494. doi:10.1519/JSC.0b013e31828f8202

Conceição, M. S., Libardi, C. A., Nogueira, F. R. D., et al. (2012). Effects of eccentric exercise on systemic concentrations of pro- and anti-inflammatory cytokines and prostaglandin (E2): comparison between young and postmenopausal women. *European journal of applied physiology, 112*(9), 3205-3213. doi:10.1007/s00421-011-2292-6

Cooke, M. B., Rybalka, E., Stathis, C. G., et al. (2010). Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. *Journal of the International Society of Sports Nutrition, 7*. doi:10.1186/1550-2783-7-30

Cooke, M. B., Rybalka, E., Stathis, C. G., et al. (2010). Whey protein isolate attenuates strength decline after eccentrically-induced muscle damage in healthy individuals. *Journal of the International Society of Sports Nutrition, 7*(1), 30. doi:10.1186/1550-2783-7-30

Cooper, R. N., Tajbakhsh, S., Mouly, V., et al. (1999). In vivo satellite cell activation via Myf5 and MyoD in regenerating mouse skeletal muscle. *J Cell Sci, 112 (Pt 17)*, 2895-2901.

Cornelison, D. D. & Wold, B. J. (1997). Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. *Dev Biol, 191*(2), 270-283. doi:10.1006/dbio.1997.8721

Cornish, S. M., Chase, J. E., Bugera, E. M., et al. (2018). Systemic IL-6 and myoglobin response to three different resistance exercise intensities in older men. *26*(3), 451. doi:10.1123/japa.2017-0167 10.1123/japa.2017-0167

Correa, C. S., Teixeira, B. C., Cobos, R. C. R., et al. (2015). High-volume resistance training reduces postprandial lipaemia in postmenopausal women. *Journal of Sports Sciences, 33*(18), 1890-1901. doi:10.1080/02640414.2015.1017732

Côté, C. H., Tremblay, M. H., Duchesne, E., et al. (2008). Inflammation-induced leukocyte accumulation in injured skeletal muscle: Role of mast cells. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 37*(6), 754-763.

Cowley, E. S., Olenick, A. A., McNulty, K. L., et al. (2021). "Invisible sportswomen": the sex data gap in sport and exercise science research. *Women in Sport and Physical Activity Journal*, 1-6. doi:10.1123/wspaj.2021-0028

Coyne, M. D., Kesick, C. M., Doherty, T. J., et al. (2000). Circadian rhythm changes in core temperature over the menstrual cycle: method for noninvasive monitoring. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 279*(4), R1316-R1320. doi:10.1152/ajpregu.2000.279.4.R1316

Crameri, R. M., Aagaard, P., Qvortrup, K., et al. (2007). Myofibre damage in human skeletal muscle: Effects of electrical stimulation versus voluntary contraction. *Journal of Physiology, 583*(1), 365-380. doi:10.1113/jphysiol.2007.128827

Crameri, R. M., Langberg, H., Magnusson, P., et al. (2004). Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *The Journal of Physiology, 558*(1), 333-340.

Creswell, J. W. (2009). Research design: Qualitative and mixed methods approaches. *London and Thousand Oaks: Sage Publications*.

Cribb, P. J. & Hayes, A. (2006). Effects of supplement-timing and resistance exercise on skeletal muscle hypertrophy. *Medicine & Science in Sports & Exercise, 38*(11), 1918-1925.

Croisier, J-L., Foidart-Dessalle, M., Tinant, F., et al. (2007). An isokinetic eccentric programme for the management of chronic lateral epicondylar tendinopathy. *British journal of sports medicine, 41*(4), 269-275.

Croisier, J-L., Forthomme, B., Namurois, M-H., et al. (2002). Hamstring muscle strain recurrence and strength performance disorders. *The American journal of sports medicine, 30*(2), 199-203.

Crotty, M. J. (1998). The foundations of social research: Meaning and perspective in the research process. *The foundations of social research*, 1-256.

Dale, M. J., Thomson, R., Coates, A., et al. (2015). Protein hydrolysates and recovery of muscle damage following eccentric exercise. *Functional Foods in Health and Disease, 5*(1), 34-43.

Dalgaard, L. B., Dalgas, U., Andersen, J. L., et al. (2019). Influence of oral contraceptive use on adaptations to resistance training. *Frontiers in Physiology, 10*(824). doi:10.3389/fphys.2019.00824

Damas, F., Libardi, C. A. & Ugrinowitsch, C. (2018). The development of skeletal muscle hypertrophy through resistance training: the role of muscle damage and muscle protein synthesis. *European journal of applied physiology, 118*(3), 485-500. doi:10.1007/s00421-017-3792-9

Damas, F., Nosaka, K., Libardi, C. A., et al. (2016$_a$). Susceptibility to exercise-induced muscle damage: a cluster analysis with a large sample. *Int J Sports Med, 37*(8), 633-640. doi:10.1055/s-0042-100281

Damas, F., Phillips, S., Vechin, F. C., et al. (2015). A review of resistance training-induced changes in skeletal muscle protein synthesis and their contribution to hypertrophy. *Sports Medicine, 45*(6), 801-807. doi:10.1007/s40279-015-0320-0

Damas, F., Phillips, S. M., Libardi, C. A., et al. (2016$_b$). Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage. *Journal of Physiology-London, 594*(18), 5209-5222. doi:10.1113/jp272472

Dannecker, E. A., Liu, Y., Rector, R. S., et al. (2012). Sex differences in exercise-induced muscle pain and muscle damage. *The journal of pain : official journal of the American Pain Society, 13*(12), 1242-1249. doi:10.1016/j.jpain.2012.09.014

Dartnall, T. J., Nordstrom, M. A. & Semmler, J. G. (2011). Adaptations in biceps brachii motor unit activity after repeated bouts of eccentric exercise in elbow flexor muscles. *Journal of neurophysiology, 105*(3), 1225-1235.

Davies, R. W., Carson, B. P. & 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*(2), 221. Retrieved from https://www.mdpi.com/2072-6643/10/2/221

De Winter, J. C., Gosling, S. D. & Potter, J. (2016). Comparing the Pearson and Spearman correlation coefficients across distributions and sample sizes: A tutorial using simulations and empirical data. *Psychological methods*, *21*(3), 273.

Deising, S., Weinkauf, B., Blunk, J., et al. (2012). NGF-evoked sensitization of muscle fascia nociceptors in humans. *Pain, 153*(8), 1673-1679. doi:10.1016/j.pain.2012.04.033

Delmonico, M. J., Kostek, M. C., Doldo, N. A., et al. (2005). Effects of moderate-velocity strength training on peak muscle power and movement velocity: do women respond differently than men? *Journal of Applied Physiology, 99*(5), 1712-1718. doi:10.1152/japplphysiol.01204.2004

Demers, L. M., Harrison, T. S., Halbert, D. R., et al. (1981). Effect of prolonged exercise on plasma prostaglandin levels. *Prostaglandins and medicine, 6*(4), 413-418.

Dieli-Conwright, C. M., Spektor, T. M., Rice, J. C., et al. (2009). Hormone therapy attenuates exercise-induced skeletal muscle damage in postmenopausal women. *Journal of Applied Physiology, 107*(3), 853-858. doi:10.1152/japplphysiol.00404.2009

Dobson, N. (2021). The effect of low-load resistance training on skeletal muscle hypertrophy in trained men: a critically appraised topic. *Journal of Sport Rehabilitation, 1*(aop), 1-6.

Donnelly, A. E., Maughan, R. J. & Whiting, P. H. (1990). Effects of ibuprofen on exercise-induced muscle soreness and indices of muscle damage. *British journal of sports medicine, 24*(3), 191-195. doi:10.1136/bjsm.24.3.191

Doriguzzi, C., Mongini, T., Palmucci, L., et al. (1984). Quantitative analysis of quadriceps muscle biopsy: Results in 30 healthy females. *Journal of the neurological sciences, 66*(2-3), 319-326.

Draganidis, D., Chatzinikolaou, A., Jamurtas, A. Z., et al. (2013). The time-frame of acute resistance exercise effects on football skill performance: The impact of exercise intensity. *Journal of Sports Sciences, 31*(7), 714-722.

Draganidis, D., Chondrogianni, N., Chatzinikolaou, A., et al. (2017). Protein ingestion preserves proteasome activity during intense aseptic inflammation and facilitates skeletal muscle recovery in humans. *Br J Nutr, 118*(3), 189-200. doi:10.1017/s0007114517001829

Dreyer, H. C., Blanco, C. E., Sattler, F. R., et al. (2006). Satellite cell numbers in young and older men 24 hours after eccentric exercise. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 33*(2), 242-253.

Dreyer, H. C., Drummond, M. J., Pennings, B., et al. (2008). Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *American Journal of Physiology-Endocrinology and Metabolism, 294*(2), E392-E400. doi:10.1152/ajpendo.00582.2007

Dreyer, H. C., Fujita, S., Cadenas, J. G., et al. (2006). Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *The Journal of Physiology, 576*(2), 613-624. doi:10.1113/jphysiol.2006.113175

Dreyer, H. C., Fujita, S., Glynn, E. L., et al. (2010). Resistance exercise increases leg muscle protein synthesis and mTOR signalling independent of sex. *Acta physiologica, 199*(1), 71-81.

Drummond, M. J., Fry, C. S., Glynn, E. L., et al. (2009). Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *The Journal of Physiology, 587*(7), 1535-1546. doi:10.1113/jphysiol.2008.163816

Drummond, M. J. & Rasmussen, B. B. (2008). Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Current opinion in clinical nutrition and metabolic care, 11*(3), 222-226. doi:10.1097/MCO.0b013e3282fa17fb

Duchen, M. R. (2004). Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Molecular aspects of medicine, 25*(4), 365-451.

Duncan, C. & Jackson, M. (1987). Different mechanisms mediate structural changes and intracellular enzyme efflux following damage to skeletal muscle. *Journal of cell science, 87*(1), 183-188.

Dupuy, O., Douzi, W., Theurot, D., et al. (2018). An evidence-based approach for choosing post-exercise recovery techniques to reduce markers of muscle damage, soreness, fatigue, and inflammation: a systematic review with meta-analysis. *Frontiers in Physiology, 9*(403). doi:10.3389/fphys.2018.00403

Duren, D. L., Sherwood, R. J., Czerwinski, S. A., et al. (2008). Body composition methods: comparisons and interpretation. *Journal of diabetes science and technology, 2*(6), 1139-1146.

Dwyer, M. K., Boudreau, S. N., Mattacola, C. G., et al. (2010). Comparison of lower extremity kinematics and hip muscle activation during rehabilitation tasks between sexes. *J Athl Train, 45*(2), 181-190. doi:10.4085/1062-6050-45.2.181

Ebbeling, C. B. & Clarkson, P. M. (1989). Exercise-induced muscle damage and adaptation. *Sports Medicine, 7*(4), 207-234.

Edwards, P., Clarke, M., DiGuiseppi, C., et al. (2002). Identification of randomized controlled trials in systematic reviews: accuracy and reliability of screening records. *Statistics in medicine, 21*(11), 1635-1640. doi:https://doi.org/10.1002/sim.1190

Edwards, R. H., Hill, D. K., Jones, D. A., et al. (1977). Fatigue of long duration in human skeletal muscle after exercise. *J Physiol, 272*(3), 769-778. doi:10.1113/jphysiol.1977.sp012072

Elkins, M. R., Herbert, R. D., Moseley, A. M., et al. (2010). Invited Commentary:Rating the Quality of Trials in Systematic Reviews of Physical Therapy Interventions. *Cardiopulmonary Physical Therapy Journal, 21*(3), 20-26.

Elliott-Sale, K. J., McNulty, K. L., Ansdell, P., et al. (2020). The effects of oral contraceptives on exercise performance in women: a systematic review and meta-analysis. *Sports Medicine*. doi:10.1007/s40279-020-01317-5

Elliott-Sale, K. J., Minahan, C. L., de Jonge, X. A. K. J., et al. (2021). Methodological considerations for studies in sport and exercise science with women as participants: a working guide for standards of practice for research on women. *Sports Medicine, 51*(5), 843-861. doi:10.1007/s40279-021-01435-8

Enns, D. L., Iqbal, S. & Tiidus, P. M. (2008). Oestrogen receptors mediate oestrogen-induced increases in post-exercise rat skeletal muscle satellite cells. *Acta physiologica, 194*(1), 81-93. doi:https://doi.org/10.1111/j.1748-1716.2008.01861.x

Enns, D. L. & Tiidus, P. M. (2008). Estrogen influences satellite cell activation and proliferation following downhill running in rats. *Journal of Applied Physiology, 104*(2), 347-353.

Eriksson, A., Tuominen, J., Valle, T., et al. (1998). Aerobic endurance exercise or circuit-type resistance training for individuals with impaired glucose tolerance? *Hormone and Metabolic Research, 30*(01), 37-41.

Eriksson, A., Lindström, M., Carlsson, L., et al. (2006). Hypertrophic muscle fibers with fissures in power-lifters; fiber splitting or defect regeneration? *Histochem Cell Biol, 126*(4), 409-417. doi:10.1007/s00418-006-0176-3

Ertel, K. A., Hallam, J. E. & Hillman, A. R. (2020). The effects of training status and exercise intensity on exercise-induced muscle damage. *The Journal of sports medicine and physical fitness, 60*(3), 449-455. doi:10.23736/s0022-4707.19.10151-x

Esmarck, B., Andersen, J. L., Olsen, S., et al. (2001). Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *The Journal of Physiology, 535*(1), 301-311. doi:https://doi.org/10.1111/j.1469-7793.2001.00301.x

Essen-Gustavsson, B. & Borges, O. (1986). Histochemical and metabolic characteristics of human skeletal muscle in relation to age. *Acta Physiologica Scandinavica, 126*(1), 107-114. doi:10.1111/j.1748-1716.1986.tb07793.x

Eston, R. G., Lemmey, A. B., McHugh, P., et al. (2000). Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scandinavian Journal of Medicine & Science in Sports, 10*(4), 199-204. doi:https://doi.org/10.1034/j.1600-0838.2000.010004199.x

Eston, R. G., Rowlands, A. V., Coulton, D., et al. (2007). Effect of flexibility training on symptoms of exercise-induced muscle damage: a preliminary study. *J Exerc Sci Fit, 5*(1), 33-39.

Evangelista, R., Pereira, R., Hackney, A. C., et al. (2011). Rest interval between resistance exercise sets: length affects volume but not creatine kinase activity or muscle soreness. *6*(1), 118. doi:10.1123/ijspp.6.1.118

Evans, W. J. & Cannon, J. G. (1991). The metabolic effects of exercise-induced muscle damage. *Exerc Sport Sci Rev, 19*, 99-125.

Farup, J., Rahbek, S. K., Knudsen, I. S., et al. (2014$_a$). Whey protein supplementation accelerates satellite cell proliferation during recovery from eccentric exercise. *Amino Acids, 46*(11), 2503-2516. doi:10.1007/s00726-014-1810-3

Fehér, A., Gazdecki, M., Véha, M., et al. (2020). A comprehensive review of the benefits of and the barriers to the switch to a plant-based diet. *Sustainability, 12*(10), 4136.

Fehring, R. J., Schneider, M. & Raviele, K. (2006). Variability in the phases of the menstrual cycle. *Journal of Obstetric, Gynecologic & Neonatal Nursing, 35*(3), 376-384. doi:https://doi.org/10.1111/j.1552-6909.2006.00051.x

Ferguson-Stegall, L., McCleave, E. L., Ding, Z., et al. (2011). Postexercise carbohydrate–protein supplementation improves subsequent exercise performance and intracellular signaling for protein synthesis. *The Journal of Strength & Conditioning Research, 25*(5), 1210-1224. doi:10.1519/JSC.0b013e318212db21

Fernandes, J. F. T., Lamb, K. L. & Twist, C. (2020). Low body fat does not influence recovery after muscle-damaging lower-limb plyometrics in young male team sport athletes. *Journal of Functional Morphology and Kinesiology, 5*(4), 79. doi:10.3390/jfmk5040079

Fernandes, T. L., Pedrinelli, A. & Hernandez, A. J. (2015). Muscle injury - physiopathology, diagnosis, treatment and clinical presentation. *Revista brasileira de ortopedia*, *46*(3), 247–255. https://doi.org/10.1016/S2255-4971(15)30190-7

Fernandez-Gonzalo, R., Lundberg, T. R., Alvarez-Alvarez, L., et al. (2014). Muscle damage responses and adaptations to eccentric-overload resistance exercise in men and women. *European journal of applied physiology, 114*(5), 1075-1084. doi:10.1007/s00421-014-2836-7

Field, A. C., Hill, C. & Lamb, G. D. (1988). Asymmetric charge movement and calcium currents in ventricular myocytes of neonatal rat. *The Journal of Physiology, 406*(1), 277-297.

Fielding, R. A., Manfredi, T. J., Ding, W., et al. (1993). Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *Am J Physiol, 265*(1 Pt 2), R166-172. doi:10.1152/ajpregu.1993.265.1.R166

Fink, J., Kikuchi, N. & Nakazato, K. (2018). Effects of rest intervals and training loads on metabolic stress and muscle hypertrophy. *Clinical Physiology and Functional Imaging, 38*(2), 261-268. doi:10.1111/cpf.12409

Fisher, J. P. & Steele, J. (2017). Heavier and lighter load resistance training to momentary failure produce similar increases in strength with differing degrees of discomfort. *Muscle & Nerve, 56*(4), 797-803. doi:https://doi.org/10.1002/mus.25537

Flacco, L., Colozzi, A., Ripari, P., et al. (1989). Dantrolene sodium in traumatic muscle contracture: double-blind clinical and pharmacological trial. *Clinical therapeutics, 11*(5), 623-632.

Fleck, S. J. (1988). Cardiovascular adaptations to resistance training. *Medicine and science in sports and exercise, 20*(5 Suppl), S146-151.

Fleischman, D. S., Navarrete, C. D. & Fessler, D. M. T. (2010). Oral contraceptives suppress ovarian hormone production. *Psychological Science, 21*(5), 750-752. Retrieved from http://www.jstor.org/stable/41062280

Fochi, A. G., Damas, F., Berton, R., et al. (2016). Greater eccentric exercise-induced muscle damage by large versus small range of motion with the same end-point. *Biology of Sport, 33*(3), 285-289. doi:10.5604/20831862.1208480

Foley, J. M., Jayaraman, R. C., Prior, B. M., et al. (1999). MR measurements of muscle damage and adaptation after eccentric exercise. *Journal of Applied Physiology, 87*(6), 2311-2318. doi:10.1152/jappl.1999.87.6.2311

Folland, J. P. & Williams, A. G. (2007). Morphological and neurological contributions to increased strength. *Sports Medicine, 37*(2), 145-168. doi:10.2165/00007256-200737020-00004

Fonseca-Alaniz, M. H., Takada, J., Alonso-Vale, M. I., et al. (2007). Adipose tissue as an endocrine organ: from theory to practice. *J Pediatr (Rio J), 83*(5 Suppl), S192-203. doi:10.2223/jped.1709

Fouré, A. & Bendahan, D. (2017). Is branched-chain amino acids supplementation an efficient nutritional strategy to alleviate skeletal muscle damage? a systematic review. *Nutrients, 9*(10), 1047.

Franchi, M. V., Atherton, P. J., Reeves, N. D., et al. (2014). Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta physiologica, 210*(3), 642-654.

Franco, C. M. D. C., Carneiro, M. A. D. S., Alves, L. T. H., et al. (2019). Lower-load is more effective than higher-load resistance training in increasing muscle mass in young

women. *The Journal of Strength & Conditioning Research, 33*, S152-S158. doi:10.1519/jsc.0000000000002970

Fredsted, A., Clausen, T. & Overgaard, K. (2008). Effects of step exercise on muscle damage and muscle Ca2+ content in men and women. *J Strength Cond Res, 22*(4), 1136-1146. doi:10.1519/JSC.0b013e318173db9b

Frei, B. (1994). Reactive oxygen species and antioxidant vitamins: mechanisms of action. *The American journal of medicine, 97*(3), S5-S13.

Fridén, J., Seger, J., Sjöström, M., et al. (1983). Adaptive response in human skeletal muscle subjected to prolonged eccentric training. *Int J Sports Med, 4*(3), 177-183. doi:10.1055/s-2008-1026031

Friden, J., Sjöström, M. & Ekblom, B. (1983). Myofibrillar damage following intense eccentric exercise in man. *International journal of sports medicine, 4*(03), 170-176.

Frontera, W. R. & Ochala, J. (2015). Skeletal Muscle: A brief review of structure and function. *Calcified Tissue International, 96*(3), 183-195. doi:10.1007/s00223-014-9915-y

Gabriel, D. A., Kamen, G. & Frost, G. (2006). Neural adaptations to resistive exercise. *Sports Medicine, 36*(2), 133-149. doi:10.2165/00007256-200636020-00004

Gayraud-Morel, B., Chrétien, F., Flamant, P., et al. (2007). A role for the myogenic determination gene Myf5 in adult regenerative myogenesis. *Developmental biology, 312*(1), 13-28.

Gee, T. I., Woolrich, T. J. & Smith, M. F. (2019). Effectiveness of whey protein hydrolysate and milk-based formulated drinks on recovery of strength and power following acute resistance exercise. *Journal of Human Kinetics, 68*(1), 193-202.

Gentil, P., Del Vecchio, F. B., Paoli, A., et al. (2017). Isokinetic dynamometry and 1RM tests produce conflicting results for assessing alterations in muscle strength. *Journal of Human Kinetics, 56*, 19–27. doi.org/10.1515/hukin-2017-0019

Gibson, W., Arendt-Nielsen, L., Taguchi, T., et al. (2009). Increased pain from muscle fascia following eccentric exercise: animal and human findings. *Experimental Brain Research, 194*(2), 299. doi:10.1007/s00221-008-1699-8

Gissel, H. (2005). The role of Ca2+ in muscle cell damage. *Ann N Y Acad Sci, 1066*, 166-180. doi:10.1196/annals.1363.013

Glenmark, B., Hedberg, G. & Jansson, E. (1992). Changes in muscle fibre type from adolescence to adulthood in women and men. *Acta Physiologica Scandinavica, 146*(2), 251-259. doi:10.1111/j.1748-1716.1992.tb09414.x

Goldfarb, A. H., Bloomer, R. J. & McKenzie, M. J. (2005). Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Medicine and science in sports and exercise, 37*(2), 234-239. doi:10.1249/01.mss.0000152887.87785.be

Gollnick, P. D., Armstrong, R. B., Saubert 4th, C. W., et al. (1972). Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *Journal of Applied Physiology, 33*(3), 312-319.

Gomes, A. V., Potter, J. D. & Szczesna-Cordary, D. (2002). The role of troponins in muscle contraction. *IUBMB life, 54*(6), 323-333.

Goodman, M. N. (1987). Differential effects of acute changes in cell Ca2+ concentration on myofibrillar and non-myofibrillar protein breakdown in the rat extensor digitorum longus muscle in vitro. Assessment by production of tyrosine and Nτ-methylhistidine. *Biochemical Journal, 241*(1), 121-127.

Gordon, A. M., Huxley, A. F. & Julian, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *The Journal of Physiology, 184*(1), 170-192. doi:10.1113/jphysiol.1966.sp007909

Gorissen, S., Crombag, J. J. R., Senden, J. M. G., et al. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids, 50*(12), 1685-1695. doi:10.1007/s00726-018-2640-5

Gorissen, S., Horstman, A. M., Franssen, R., et al. (2016). Ingestion of wheat protein increases in vivo muscle protein synthesis rates in healthy older men in a randomized trial. *The Journal of nutrition, 146*(9), 1651-1659. doi:10.3945/jn.116.231340

Green, H., Goreham, C., Ouyang, J., et al. (1999). Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 276*(2), R591-R596. doi:10.1152/ajpregu.1999.276.2.R591

Greendale, G. A., Sternfeld, B., Huang, M., et al. (2019). Changes in body composition and weight during the menopause transition. *JCI insight, 4*(5). doi:10.1172/jci.insight.124865

Grgic, J., Lazinica, B., Mikulic, P., et al. (2017). The effects of short versus long inter-set rest intervals in resistance training on measures of muscle hypertrophy: A systematic review. *European Journal of Sport Science, 17*(8), 983-993.

Grgic, J., Lazinica, B., Schoenfeld, B. J., et al. (2020). Test–retest reliability of the one-repetition maximum (1rm) strength assessment: a systematic review. *Sports Medicine - Open, 6*(1), 31. doi:10.1186/s40798-020-00260-z

Grijalba, M. T., Vercesi, A. E. & Schreier, S. (1999). Ca2+-induced increased lipid packing and domain formation in submitochondrial particles. A possible early step in the mechanism of Ca2+-stimulated generation of reactive oxygen species by the respiratory chain. *Biochemistry, 38*(40), 13279-13287.

Grubic, T. J., Sowinski, R. J., Nevares, B. E., et al. (2019). Comparison of ingesting a food bar containing whey protein and isomalto-oligosaccharides to carbohydrate on performance and recovery from an acute bout of resistance-exercise and sprint conditioning: an open label, randomized, counterbalanced, crossover pilot study. *Journal of the International Society of Sports Nutrition, 16*(1).

Grucza, R., Pekkarinen, H., Titov, E-K., et al. (1993). Influence of the menstrual cycle and oral contraceptives on thermoregulatory responses to exercise in young women. *European Journal of Applied Physiology and Occupational Physiology, 67*(3), 279-285. doi:10.1007/BF00864229

Guba, E. G. & Lincoln, Y. S. (1994). Competing paradigms in qualitative research. *Handbook of qualitative research, 2*(163-194), 105.

Güzel, N. A., Hazar, S. & Erbas, D. (2007). Effects of different resistance exercise protocols on nitric oxide, lipid peroxidation and creatine kinase activity in sedentary males. *Journal of sports science & medicine, 6*(4), 417-422. Retrieved from https://pubmed.ncbi.nlm.nih.gov/24149472

Hackney, A. C., McCracken-Compton, M. A. & Ainsworth, B. (1994). Substrate responses to submaximal exercise in the midfollicular and midluteal phases of the menstrual cycle. *International Journal of Sport Nutrition, 4*(3), 299-308. doi:10.1123/ijsn.4.3.299

Hall, E. C. R., Semenova, E. A., Bondareva, E. A., et al. (2021). Association of muscle fiber composition with health and exercise-related traits in athletes and untrained subjects. *Biology of Sport, 38*(4), 659-666. doi:10.5114/biolsport.2021.102923

Hansen, M. (2018). Female hormones: do they influence muscle and tendon protein metabolism? *Proc Nutr Soc, 77*(1), 32-41. doi:10.1017/s0029665117001951

Hansen, M., Koskinen, S. O., Petersen, S. G., et al. (2008). Ethinyl oestradiol administration in women suppresses synthesis of collagen in tendon in response to exercise. *The Journal of Physiology, 586*(12), 3005-3016.

Hansen, M., Langberg, H., Holm, L., et al. (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*(1), 62-72.

Hansen, M., Miller, B. F., Holm, L., et al. (2009). Effect of administration of oral contraceptives in vivo on collagen synthesis in tendon and muscle connective tissue in young women. *Journal of Applied Physiology, 106*(4), 1435-1443. doi:10.1152/japplphysiol.90933.2008

Hariton, E. & Locascio, J. J. (2018). Randomised controlled trials - the gold standard for effectiveness research: Study design: randomised controlled trials. *BJOG : an international journal of obstetrics and gynaecology, 125*(13), 1716-1716. doi:10.1111/1471-0528.15199

Hartman, J. W., Tang, J. E., Wilkinson, S. B., et al. (2007). Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *The American journal of clinical nutrition, 86*(2), 373-381.

Harty, P. S., Cottet, M. L., Malloy, J. K., et al. (2019). Nutritional and supplementation strategies to prevent and attenuate exercise-induced muscle damage: a brief review. *Sports Medicine - Open, 5*(1), 1. doi:10.1186/s40798-018-0176-6

Hasegawa, Y., Mekata, Y., Ayaka, S., et al. (2014). Effect of egg white protein supplementation prior to acute resistance training on muscle damage indices in untrained japanese men. *Montenegrin Journal of Sports Science & Medicine, 3*(2), 5-12.

Hasenoehrl, T., Wessner, B., Tschan, H., et al. (2017). Eccentric resistance training intensity may affect the severity of exercise induced muscle damage. *The Journal of sports medicine and physical fitness, 57*(9), 1195-1204. doi:10.23736/s0022-4707.16.06476-8

Hather, B. M., Tesch, P. A., Buchanan, P., et al. (1991). Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiologica Scandinavica, 143*(2), 177-185. doi:10.1111/j.1748-1716.1991.tb09219.x

Hazel, J. & Williams, E. (1990). The role of alterations in membrane lipid compositon in enabling physiological adaptation of organisms to their physical environment. *Progress in lipid research, 29*, 167-227. doi:10.1016/0163-7827(90)90002-3

Heavens, K. R., Szivak, T. K., Hooper, D. R., et al. (2014). The effects of high intensity short rest resistance exercise on muscle damage markers in men and women. *J Strength Cond Res, 28*(4), 1041-1049. doi:10.1097/jsc.0000000000000236

Hector, A. J. & Phillips, S. M. (2018). Protein recommendations for weight loss in elite athletes: a focus on body composition and performance. *28*(2), 170.

doi:10.1123/ijsnem.2017-0273 10.1123/ijsnem.2017-0273 10.1123/ijsnem.2017-0273 10.1123/ijsnem.2017-0273

Heled, Y., Bloom, M. S., Wu, T. J., et al. (2007). CM-MM and ACE genotypes and physiological prediction of the creatine kinase response to exercise. *Journal of Applied Physiology, 103*(2), 504-510.

Henneman, E., Somjen, G. & Carpenter, D. O. (1965). Functional significance of cell size in spinal motoneurons. *Journal of neurophysiology, 28*(3), 560-580.

Hesselink, M. K., Kuipers, H., Geurten, P., et al. (1996). Structural muscle damage and muscle strength after incremental number of isometric and forced lengthening contractions. *Journal of Muscle Research & Cell Motility, 17*(3), 335-341.

Hickner, R., Mehta, P., Dyck, D., et al. (2001). Relationship between fat-to-fat-free mass ratio and decrements in leg strength after downhill running. *Journal of applied physiology, 90*, 1334-1341. doi:10.1152/jappl.2001.90.4.1334

Hicks, K. M., Onambélé, G., Winwood, K., et al. (2016). Muscle damage following maximal eccentric knee extensions in males and females. *PLoS One, 11*(3), e0150848.

Hicks, K. M., Onambélé-Pearson, G., Winwood, K., et al. (2017). Oral contraceptive pill use and the susceptibility to markers of exercise-induced muscle damage. *European journal of applied physiology, 117*(7), 1393-1402. doi:10.1007/s00421-017-3629-6

Higgins, J. P., Thompson, S. G., Deeks, J. J., et al. (2003). Measuring inconsistency in meta-analyses. *Bmj, 327*(7414), 557-560.

Highstead, R. G., Tipton, K. D., Creson, D. L., et al. (2005). Incidence of associated events during the performance of invasive procedures in healthy human volunteers. *Journal of Applied Physiology, 98*(4), 1202-1206. doi:10.1152/japplphysiol.01076.2004

Hill, A. V. (1925). Length of muscle, and the heat and tension developed in an isometric contraction. *The Journal of Physiology, 60*(4), 237-263. doi:10.1113/jphysiol.1925.sp002242

Hind, K., Oldroyd, B. & Truscott, J. G. (2011). In vivo precision of the GE Lunar iDXA densitometer for the measurement of total body composition and fat distribution in adults. *European Journal of Clinical Nutrition, 65*(1), 140-142. doi:10.1038/ejcn.2010.190

Hind, K., Slater, G., Oldroyd, B., et al. (2018). Interpretation of dual-energy x-ray absorptiometry-derived body composition change in athletes: a review and recommendations for best practice. *Journal of Clinical Densitometry, 21*(3), 429-443. doi:https://doi.org/10.1016/j.jocd.2018.01.002

Hind, K. (2022). Application of dual energy X-ray absorptiometry. In *Sport and Exercise Physiology Testing Guidelines* (pp. 167-181): Routledge.

Hirose, L., Sato, M., Yanagisawa, O., et al. (2013). Milk peptide intake may decrease muscle damage after eccentric exercise. *International Journal of Sport and Health Science, 11*, 20-28. doi:10.5432/ijshs.201223

Hirose, L., Nosaka, K., Newton, M., et al. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exercise immunology review, 10*, 75-90.

Hisaeda, H., Miyagawa, K., Kuno, S-Y., et al. (1996). Influence of two different modes of resistance training in female subjects. *Ergonomics, 39*(6), 842-852. doi:10.1080/00140139608964505

Hjermstad, M. J., Fayers, P. M., Haugen, D. F., et al. (2011). Studies comparing numerical rating scales, verbal rating scales, and visual analogue scales for assessment of pain intensity in adults: a systematic literature review. *Journal of Pain and Symptom Management, 41*(6), 1073-1093. doi.org/10.1016/j.jpainsymman.2010.08.016

Hody, S., Croisier, J-L., Bury, T., et al. (2019). Eccentric muscle contractions: risks and benefits. *Frontiers in Physiology, 10*, 536.

Hoffman, J. R., Ratamess, N. A., Tranchina, C. P., et al. (2009). Effect of protein-supplement timing on strength, power, and body-composition changes in resistance-trained men. *International journal of sport nutrition and exercise metabolism, 19*(2), 172-185.

Hoffman, J. R., Ratamess, N. A., Tranchina, C. P., et al. (2010). Effect of a proprietary protein supplement on recovery indices following resistance exercise in strength/power athletes. *Amino Acids, 38*(3), 771-778. doi:10.1007/s00726-009-0283-2

Holloszy, J. O. & Booth, F. W. (1976). Biochemical adaptations to endurance exercise in muscle. *Annual review of physiology, 38*(1), 273-291.

Holwerda, A. M., Kouw, I. W., Trommelen, J., et al. (2016). Physical activity performed in the evening increases the overnight muscle protein synthetic response to presleep protein ingestion in older men. *The Journal of nutrition, 146*(7), 1307-1314.

Holwerda, A. M., Paulussen, K. J. M., Overkamp, M., et al. (2019). Dose-dependent increases in whole-body net protein balance and dietary protein-derived amino acid incorporation into myofibrillar protein during recovery from resistance exercise in older men. *The Journal of nutrition, 149*(2), 221-230. doi:10.1093/jn/nxy263

Honour, J. W. (2018). Biochemistry of the menopause. *Ann Clin Biochem, 55*(1), 18-33. doi:10.1177/0004563217739930

Hostler, D., Crill, M. T., Hagerman, F. C., et al. (2001). The effectiveness of 0.5-lb increments in progressive resistance exercise. *Journal of Strength and Conditioning Research, 15*(1), 86-91.

Hough, T. (1902). Ergographic studies in muscular soreness. *American physical education review, 7*(1), 1-17.

House, E. R. (1991). Realism in research. *Educational researcher, 20*(6), 2-9.

Howatson, G., Van Someren, K. & Hortobagyi, T. (2007). Repeated bout effect after maximal eccentric exercise. *International journal of sports medicine, 28*(07), 557-563.

Howatson, G. & Van Someren, K. A. (2003). Ice massage: effects on exercise-induced muscle damage. *Journal of Sports Medicine and Physical Fitness, 43*(4), 500-505.

Hubal, M. J. & Clarkson, P. M. (2009). Counterpoint: estrogen and sex do not significantly influence post-exercise indexes of muscle damage, inflammation, and repair. *Journal of Applied Physiology, 106*(3), 1012-1014. doi:10.1152/japplphysiol.90848.2008a

Hubal, M. J. Rubinstein, S. R., & Clarkson, P. M. (2008). Muscle function in men and women during maximal eccentric exercise. *The Journal of Strength & Conditioning Research, 22*(4), 1332-1338. doi:10.1519/JSC.0b013e31817392ec

Hunt, J. E. A., Coelho, M. O. C., Buxton, S., et al. (2021). Consumption of new zealand blackcurrant extract improves recovery from exercise-induced muscle damage in non-resistance trained men and women: a double-blind randomised trial. *Nutrients, 13*(8), 2875.

Hurley, C. F., Hatfield, D. L. & Riebe, D. A. (2013). The effect of caffeine ingestion on delayed onset muscle soreness. *J Strength Cond Res, 27*(11), 3101-3109. doi:10.1519/JSC.0b013e3182a99477

Huxley, H. & Hanson, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature, 173*(4412), 973-976.

Hyldahl, R. D., Chen, T. C. & Nosaka, K. (2017). Mechanisms and mediators of the skeletal muscle repeated bout effect. *Exerc Sport Sci Rev, 45*(1), 24-33. doi:10.1249/jes.0000000000000095

Hyldahl, R. D., Nelson, B., Xin, L., et al. (2015). Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *The FASEB Journal, 29*(7), 2894-2904. doi:https://doi.org/10.1096/fj.14-266668

Ingalls, C. P., Warren, G. L., Williams, J. H., et al. (1998). E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *J Appl Physiol (1985), 85*(1), 58-67. doi:10.1152/jappl.1998.85.1.58

Isacco, L., Duché, P. & Boisseau, N. (2012). Influence of hormonal status on substrate utilization at rest and during exercise in the female population. *Sports Medicine, 42*(4), 327-342.

Israel, S. L. & Schneller, O. (1950). The thermogenic property of progesterone. *Obstetrical & Gynecological Survey, 5*(4), 532-533.

Ives, S. J., Bloom, S., Matias, A., et al. (2017). Effects of a combined protein and antioxidant supplement on recovery of muscle function and soreness following eccentric exercise. *Journal of the International Society of Sports Nutrition, 14*(1), 21. doi:10.1186/s12970-017-0179-6

Ivey, S. J., Tracy, B., Lemmer, J., et al. (2000). Effects of strength training and detraining on muscle quality: age and gender comparisons. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 55*(3), B152-B157.

Ivey, F. M., Roth, S. M., Ferrell, R. E., et al. (2000). Effects of age, gender, and myostatin genotype on the hypertrophic response to heavy resistance strength training. *The Journals of Gerontology: Series A, 55*(11), M641-M648. doi:10.1093/gerona/55.11.M641

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1984). Experimental skeletal muscle damage: the nature of the calcium-activated degenerative processes. *European Journal of Clinical Investigation, 14*(5), 369-374. doi:https://doi.org/10.1111/j.1365-2362.1984.tb01197.x

James, L. J., Stevenson, E. J., Rumbold, P. L., et al. (2019). Cow's milk as a post-exercise recovery drink: implications for performance and health. *European Journal of Sport Science, 19*(1), 40-48.

Jones, C., Allen, T., Talbot, J., et al. (1997). Changes in the mechanical properties of human and amphibian muscle after eccentric exercise. *Eur J Appl Physiol Occup Physiol, 76*(1), 21-31. doi:10.1007/s004210050208

Josse, A. R., Atkinson, S. A., Tarnopolsky, M. A., et al. (2011). Increased consumption of dairy foods and protein during diet- and exercise-induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women. *J Nutr, 141*(9), 1626-1634. doi:10.3945/jn.111.141028

Josse, A. R., Tang, J., Tarnopolsky, M., et al. (2009). Body composition and strength changes in women with milk and resistance exercise. *Medicine and science in sports and exercise, 42,* 1122-1130. doi:10.1249/MSS.0b013e3181c854f6

Kadi, F., Charifi, N., Denis, C., et al. (2004). Satellite cells and myonuclei in young and elderly women and men. *Muscle & Nerve, 29*(1), 120-127. doi:https://doi.org/10.1002/mus.10510

Kalia, V., Leung, D. G., Sneag, D. B., et al. (2017). Advanced MRI techniques for muscle imaging. *Seminars in musculoskeletal radiology, 21*(4), 459-469. doi:10.1055/s-0037-1604007

Kamen, G. & Knight, C. A. (2004). Training-related adaptations in motor unit discharge rate in young and older adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 59*(12), 1334-1338.

Kameyama, T. & Etlinger, J. D. (1979). Calcium-dependent regulation of protein synthesis and degradation in muscle. *Nature, 279*(5711), 344-346.

Kanda, K., Kaoru, S., Jun, S., et al. (2014). Evaluation of serum leaking enzymes and investigation into new biomarkers for exercise-induced muscle damage. *Exercise immunology review, 20,* 39-54.

Kanda, K., Sugama, K., Hayashida, H., et al. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exercise immunology review, 19,* 72-85.

Karakus, M. & Akkurt, S. (2020). The effect of use of protein supplements on muscle damage. *Progress in Nutrition, 22,* 194-198. doi:10.23751/pn.v22i1-S.9882

Karastergiou, K., Smith, S. R., Greenberg, A. S., et al. (2012). Sex differences in human adipose tissues - the biology of pear shape. *Biology of sex differences, 3*(1), 13-13. doi:10.1186/2042-6410-3-13

Karvetti, R-L. & Knuts, L-R. (1985). Validity of the 24-hour dietary recall. *Journal of the American Dietetic Association, 85*(11), 1437-1442. doi:https://doi.org/10.1016/S0002-8223(21)03818-9

Kattapong, K. R., Fogg, L. F. & Eastman, C. I. (1995). Effect of sex, menstrual cycle phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International, 12*(4), 257-266. doi:10.3109/07420529509057274

Kaviani, M., Abassi, A. & Chilibeck, P. D. (2019). Creatine monohydrate supplementation during eight weeks of progressive resistance training increases strength in as little as

two weeks without reducing markers of muscle damage. *J Sports Med Phys Fitness, 59*(4), 608-612. doi:10.23736/s0022-4707.18.08406-2

Keane, K. M., Salicki, R., Goodall, S., et al. (2015). Muscle damage response in female collegiate athletes after repeated sprint activity. *The Journal of Strength & Conditioning Research, 29*(10), 2802-2807. doi:10.1519/jsc.0000000000000961

Kekäläinen, T., Kokko, K., Sipilä, S., et al. (2018). Effects of a 9-month resistance training intervention on quality of life, sense of coherence, and depressive symptoms in older adults: randomized controlled trial. *Quality of life research, 27*(2), 455-465.

Kerksick, C., Taylor 4th, L., Harvey, A., et al. (2008). Gender-related differences in muscle injury, oxidative stress, and apoptosis. *Medicine & Science in Sports & Exercise, 40*(10), 1772-1780.

Kim, J., Lee, C. & Lee, J. (2017). Effect of timing of whey protein supplement on muscle damage markers after eccentric exercise. *Journal of Exercise Rehabilitation, 13*(4), 436-440. doi:10.12965/jer.1735034.517

Kim, J. & Lee, J. (2015). The relationship of creatine kinase variability with body composition and muscle damage markers following eccentric muscle contractions. *Journal of exercise nutrition & biochemistry, 19*(2), 123-129. doi:10.5717/jenb.2015.15061910

Kim, J. & So, W-Y. (2018). High body mass index is associated with the extent of muscle damage after eccentric exercise. *International Journal of Environmental Research and Public Health, 15*(7), 1378.

Kinser, A. M., Sands, W. A. & Stone, M. H. (2009). Reliability and validity of a pressure algometer. *The Journal of Strength & Conditioning Research, 23*(1), 312-314. doi:10.1519/JSC.0b013e31818f051c

Knoblauch, M. A., O'Connor, D. P. & Clarke, M. S. (2013). Obese mice incur greater myofiber membrane disruption in response to mechanical load compared with lean mice. *Obesity, 21*(1), 135-143.

Koenig, R. T., Dickman, J. R., Kang, C. H., et al. (2016). Avenanthramide supplementation attenuates eccentric exercise-inflicted blood inflammatory markers in women. *Eur J Appl Physiol, 116*(1), 67-76. doi:10.1007/s00421-015-3244-3

Köhne, J. L., Ormsbee, M. J. & McKune, A. J. (2016). The effects of a multi-ingredient supplement on markers of muscle damage and inflammation following downhill running in females. *Journal of the International Society of Sports Nutrition, 13*, 44-44. doi:10.1186/s12970-016-0156-5

Komulainen, J., Koskinen, S. O., Kalliokoski, R., et al. (1999). Gender differences in skeletal muscle fibre damage after eccentrically biased downhill running in rats. *Acta Physiologica Scandinavica, 165*(1), 57-63.

Konrad, M., Nieman, D. C., Henson, D. A., et al. (2011). The acute effect of ingesting a quercetin-based supplement on exercise-induced inflammation and immune changes in runners. *International journal of sport nutrition and exercise metabolism, 21*(4), 338-346.

Koopman, R., Pennings, B., Zorenc, A. H. G., et al. (2007). Protein ingestion further augments s6k1 phosphorylation in skeletal muscle following resistance type exercise in males. *The Journal of nutrition, 137*(8), 1880-1886. doi:10.1093/jn/137.8.1880

Korotkova, M. & Lundberg, I. E. (2014). The skeletal muscle arachidonic acid cascade in health and inflammatory disease. *Nat Rev Rheumatol, 10*(5), 295-303. doi:10.1038/nrrheum.2014.2

Koska, J., Blazícek, P., Marko, M., et al. (2000). Insulin, catecholamines, glucose and antioxidant enzymes in oxidative damage during different loads in healthy humans. *Physiological research, 49 Suppl 1*, S95-100.

Kouw, I. W., Holwerda, A. M., Trommelen, J., et al. (2017). Protein ingestion before sleep increases overnight muscle protein synthesis rates in healthy older men: a randomized controlled trial. *The Journal of nutrition, 147*(12), 2252-2261. doi:10.3945/jn.117.254532

Kouw, I. W., Pinckaers, P. J. M., Le Bourgot, C., et al. (2021). 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*, 1-11. doi:10.1017/S0007114521004906

Kraemer, W. J., Ratamess, N. A. & French, D. N. (2002). Resistance training for health and performance. *Current Sports Medicine Reports, 1*(3), 165-171.

Kriengsinyos, W., Wykes, L. J., Goonewardene, L. A., et al. (2004). Phase of menstrual cycle affects lysine requirement in healthy women. *American Journal of Physiology-Endocrinology and Metabolism, 287*(3), E489-E496. doi:10.1152/ajpendo.00262.2003

Kubo, K., Ikebukuro, T. & Yata, H. (2020). Effects of 4, 8, and 12 repetition maximum resistance training protocols on muscle volume and strength. *The Journal of Strength & Conditioning Research, Publish Ahead of Print*. doi:10.1519/jsc.0000000000003575

Kuo, I. Y. & Ehrlich, B. E. (2015). Signaling in muscle contraction. *Cold Spring Harb Perspect Biol, 7*(2), a006023. doi:10.1101/cshperspect.a006023

Kutáč, P. & Sigmund, M. (2017). Assessment of body composition of female volleyball players of various performance levels. *Journal of Physical Education and Sport, 17*(2), 556.

Lamb, G. D. (2000). Excitation-contraction coupling in skeletal muscle: comparisons with cardiac muscle. *Clinical and Experimental Pharmacology and Physiology, 27*(3), 216-224.

Lamb, G. D. & Walsh, T. (1987). Calcium currents, charge movement and dihydropyridine binding in fast-and slow-twitch muscles of rat and rabbit. *The Journal of Physiology, 393*(1), 595-617.

Lamb, K. L., Ranchordas, M. K., Johnson, E., et al. (2019). No effect of tart cherry juice or pomegranate juice on recovery from exercise-induced muscle damage in non-resistance trained men. *Nutrients, 11*(7), 1593.

Lamont, L. S., Lemon, P. & Bruot, B. C. (1987). Menstrual cycle and exercise effects on protein catabolism. *Medicine and science in sports and exercise, 19*(2), 106-110.

Landreneau, K. J. & Creek, W. (2009). Sampling strategies. *Available from: http://www. natco1. org*.

Lariviere, F., Moussalli, R. & Garrel, D. R. (1994). Increased leucine flux and leucine oxidation during the luteal phase of the menstrual cycle in women. *American Journal of Physiology-Endocrinology and Metabolism, 267*(3), E422-E428. doi:10.1152/ajpendo.1994.267.3.E422

LaRoche, D. P. & Connolly, D. A. J. (2006). Effects of stretching on passive muscle tension and response to eccentric exercise. *The American journal of sports medicine, 34*(6), 1000-1007. doi:10.1177/0363546505284238

Larson, H. K., McFadden, K., McHugh, T-L. F., et al. (2018). When you don't get what you want—and it's really hard: Exploring motivational contributions to exercise dropout. *Psychology of Sport and Exercise, 37*, 59-66. doi:https://doi.org/10.1016/j.psychsport.2018.04.006

Lasevicius, T., Schoenfeld, B. J., Silva-Batista, C., et al. (2019). Muscle failure promotes greater muscle hypertrophy in low-load but not in high-load resistance training. *The Journal of Strength & Conditioning Research, Publish Ahead of Print*. doi:10.1519/jsc.0000000000003454

Lastayo, P., Reich, T., Urquhart, M., et al. (1999). Chronic eccentric exercise: improvements in muscle strength can occur with little demand for oxygen. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 276*(2), R611-R615.

Lauritzen, F., Paulsen, G., Raastad, T., et al. (2009). Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. *Journal of Applied Physiology, 107*(6), 1923-1934. doi:10.1152/japplphysiol.00148.2009

Laustiola, K., Seppala, E., Nikkari, T., et al. (1984). Exercise-induced increase in plasma arachidonic acid and thromboxane b2 in healthy men: effect of β-adrenergic blockade. *Journal of Cardiovascular Pharmacology, 6*(3), 449-454.

Lautenbacher, S., Kundermann, B. & Krieg, J. C. (2006). Sleep deprivation and pain perception. *Sleep Med Rev, 10*(5), 357-369. doi:10.1016/j.smrv.2005.08.001

Lavender, A. P. & Nosaka, K. (2008). Changes in markers of muscle damage of middle-aged and young men following eccentric exercise of the elbow flexors. *Journal of Science and Medicine in Sport, 11*(2), 124-131.

Laver, D., Baynes, T. & Dulhunty, A. (1997). Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent mechanisms. *The Journal of membrane biology, 156*(3), 213-229.

Layne, J. E. & Nelson, M. E. (1999). The effects of progressive resistance training on bone density: a review. *Medicine & Science in Sports & Exercise, 31*(1), 25-30.

Le, G., Novotny, S. A., Mader, T. L., et al. (2018). A moderate oestradiol level enhances neutrophil number and activity in muscle after traumatic injury but strength recovery is accelerated. *The Journal of Physiology, 596*(19), 4665-4680.

Lee, S. Y. & Gallagher, D. (2008). Assessment methods in human body composition. *Curr Opin Clin Nutr Metab Care, 11*(5), 566-572. doi:10.1097/MCO.0b013e32830b5f23

Liberati, A., Altman, D. G., Tetzlaff, J., et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *Bmj, 339*, b2700. doi:10.1136/bmj.b2700

Lin, M-J., Nosaka, K., Ho, C-C., et al. (2018). Influence of maturation status on eccentric exercise-induced muscle damage and the repeated bout effect in females. *Frontiers in Physiology, 8*. doi:10.3389/fphys.2017.01118

Lockwood, C. M., Roberts, M. D., Dalbo, V. J., et al. (2017). Effects of hydrolyzed whey versus other whey protein supplements on the physiological response to 8 weeks of

resistance exercise in college-aged males. *Journal of the American College of Nutrition, 36*(1), 16-27. doi:10.1080/07315724.2016.1140094

Lohman, M., Tallroth, K., Kettunen, J. A., et al. (2009). Reproducibility of dual-energy x-ray absorptiometry total and regional body composition measurements using different scanning positions and definitions of regions. *Metabolism, 58*(11), 1663-1668. doi:https://doi.org/10.1016/j.metabol.2009.05.023

Longland, T. M., Oikawa, S. Y., Mitchell, C. J., et al. (2016). Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial. *The American journal of clinical nutrition, 103*(3), 738-746.

Lu, H., Huang, D., Ransohoff, R. M., et al. (2011). Acute skeletal muscle injury: CCL2 expression by both monocytes and injured muscle is required for repair. *Faseb j, 25*(10), 3344-3355. doi:10.1096/fj.10-178939

Lucy, J. (1972). Functional and structural aspects of biological membranes: a suggested structural role for vitamin E in the control of membrane permeability and stability. *Annals of the New York Academy of Sciences, 203*(1), 4-11.

Luk, H-Y., Levitt, D. E., Boyett, J. C., et al. (2019). Resistance exercise-induced hormonal response promotes satellite cell proliferation in untrained men but not in women. *American Journal of Physiology-Endocrinology and Metabolism, 317*(2), E421-E432.

Lunn, W. R., Pasiakos, S. M., Colletto, M. R., et al. (2012). Chocolate milk and endurance exercise recovery: protein balance, glycogen, and performance. *Medicine & Science in Sports & Exercise, 44*(4), 682-691.

Lynn, R. & Morgan, D. L. (1994). Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J Appl Physiol (1985), 77*(3), 1439-1444. doi:10.1152/jappl.1994.77.3.1439

Lynn, R., Talbot, J. A. & Morgan, D. L. (1998). Differences in rat skeletal muscles after incline and decline running. *J Appl Physiol (1985), 85*(1), 98-104. doi:10.1152/jappl.1998.85.1.98

Ma, F., Li, Y., Yang, J., et al. (2020). The effectiveness of low intensity exercise and blood flow restriction without exercise on exercise induced muscle damage: A systematic review. *Physical Therapy in Sport, 46*, 77-88. doi.org/10.1016/j.ptsp.2020.08.011

Macaluso, F., Isaacs, A. W., Felice, V. D., et al. (2014). Acute change of titin at mid-sarcomere remains despite 8 wk of plyometric training. *Journal of Applied Physiology, 116*(11), 1512-1519. doi:10.1152/japplphysiol.00420.2013

MacDougall, J. D., Gibala, M. J., Tarnopolsky, M. A., et al. (1995). The time course for elevated muscle protein synthesis following heavy resistance exercise. *Canadian Journal of applied physiology, 20*(4), 480-486.

Machado, M. & Willardson, J. M. (2010). Short recovery augments magnitude of muscle damage in high responders. *Medicine and science in sports and exercise, 42*(7), 1370-1374.

MacIntyre, D. L., Reid, W. D., Lyster, D. M. et al. (2000). Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. *European journal of applied physiology, 81*(1-2), 47-53.

Mackay, K., González, C., Zbinden-Foncea, H., et al. (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*(11), 2733-2744. doi:10.1007/s00421-019-04254-y

Mackey, A. L., Kjaer, M., Dandanell, S., et al. (2007). The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans. *Journal of Applied Physiology, 103*(2), 425-431. doi:10.1152/japplphysiol.00157.2007

Mackova, E., Melichna, J., Havlíčková, L., et al. (1986). Skeletal muscle characteristics of sprint cyclists and nonathletes. *International journal of sports medicine, 7*(05), 295-297.

Macnaughton, L. S., Wardle, S. L., Witard, O. C., et al. (2016). The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiol Rep, 4*(15). doi:10.14814/phy2.12893

Maher, C. G., Sherrington, C., Herbert, R. D., et al. (2003). Reliability of the PEDro scale for rating quality of randomized controlled trials. *Physical therapy, 83*(8), 713-721.

Makovey, J., Naganathan, V. & Sambrook, P. (2005). Gender differences in relationships between body composition components, their distribution and bone mineral density: a cross-sectional opposite sex twin study. *Osteoporosis International, 16*(12), 1495-1505. doi:10.1007/s00198-005-1841-4

Malm., C (2001). Exercise-induced muscle damage and inflammation: fact or fiction? *Acta Physiologica Scandinavica, 171*(3), 233-239.

Malm, C., Nyberg, P., Engstrom, M., et al. (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *The Journal of Physiology, 529 Pt 1*(Pt 1), 243-262. doi:10.1111/j.1469-7793.2000.00243.x

Malm, C., Nyberg, P., Engstrom, M., et al. (2000). Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol, 529 Pt 1*(Pt 1), 243-262. doi:10.1111/j.1469-7793.2000.00243.x

Manfredi, T. G., Fielding, R. A., O'Reilly, K. P., et al. (1991). Plasma creatine kinase activity and exercise-induced muscle damage in older men. *Medicine and science in sports and exercise, 23*(9), 1028-1034.

Mangan, G., Bombardier, E., Mitchell, A. S., et al. (2014). Oestrogen-dependent satellite cell activation and proliferation following a running exercise occurs via the PI3K signalling pathway and not IGF-1. *Acta physiologica, 212*(1), 75-85. doi:https://doi.org/10.1111/apha.12317

Marchettini, P., Simone, D. A., Caputi, G., et al. (1996). Pain from excitation of identified muscle nociceptors in humans. *Brain research, 740*(1-2), 109-116.

Margaritelis, N. V., Theodorou, A. A., Chatzinikolaou, P. N., et al. (2021). Eccentric exercise per se does not affect muscle damage biomarkers: early and late phase adaptations. *European journal of applied physiology, 121*(2), 549-559. doi:10.1007/s00421-020-04528-w

Margaritelis, N. V., Theodorou, A. A., Kyparos, A., et al. (2019). Effect of body composition on redox homeostasis at rest and in response to exercise: the case of underfat women. *Journal of Sports Sciences, 37*(14), 1630-1637.

Marqués-Jiménez, D., Calleja-González, J., Arratibel, I., et al. (2016). Are compression garments effective for the recovery of exercise-induced muscle damage? A systematic review with meta-analysis. *Physiology & Behavior, 153*, 133-148. doi:https://doi.org/10.1016/j.physbeh.2015.10.027

Martel, G. F., Roth, S. M., Ivey, F. M., et al. (2006). Age and sex affect human muscle fibre adaptations to heavy-resistance strength training. *Experimental Physiology, 91*(2), 457-464. doi:10.1113/expphysiol.2005.032771

Martin, D., Sale, C., Cooper, S. B., et al. (2018). Period prevalence and perceived side effects of hormonal contraceptive use and the menstrual cycle in elite athletes. *Int J Sports Physiol Perform, 13*(7), 926-932. doi:10.1123/ijspp.2017-0330

Martonosi, A. N. (1984). Mechanisms of Ca2+ release from sarcoplasmic reticulum of skeletal muscle. *Physiological reviews, 64*(4), 1240-1320. doi:10.1152/physrev.1984.64.4.1240

Marzolini, S., Mertens, D. J., Oh, P. I., et al. (2010). Self-reported compliance to home-based resistance training in cardiac patients. *European journal of cardiovascular prevention and rehabilitation, 17*(1), 35-49. doi:10.1097/HJR.0b013e32832da020

Maughan, R. J., Burke, L. M., Dvorak, J., et al. (2018). IOC consensus statement: dietary supplements and the high-performance athlete. *Int J Sport Nutr Exerc Metab, 28*(2), 104-125. doi:10.1123/ijsnem.2018-0020

Mavropalias, G., Calapre, L., Morici, M., et al. (2021). Changes in plasma hydroxyproline and plasma cell-free DNA concentrations after higher- versus lower-intensity eccentric cycling. *European journal of applied physiology, 121*(4), 1087-1097. doi:10.1007/s00421-020-04593-1

Mayhew, D. L., Thyfault, J. P. & Koch, A. J. (2005). Rest-interval length affects leukocyte levels during heavy resistance exercise. *Journal of Strength and Conditioning Research, 19*(1), 16.

Mazani, M., Nemati, A., Baghi, A. N., et al. (2018). The effect of probiotic yoghurt consumption on oxidative stress and inflammatory factors in young females after exhaustive exercise. *J. Pak. Med. Assoc, 68*(12), 1748-1754.

McCully, K. K. & Faulkner, J. A. (1985). Injury to skeletal muscle fibers of mice following lengthening contractions. *Journal of Applied Physiology, 59*(1), 119-126.

McCurdy, K., Langford, G. A., Cline, A. L., et al. (2004). The reliability of 1- and 3rm tests of unilateral strength in trained and untrained men and women. *J Sports Sci Med, 3*(3), 190-196.

McGinley, C., Shafat, A. & Donnelly, A. E. (2009). Does antioxidant vitamin supplementation protect against muscle damage? *Sports Medicine, 39*(12), 1011-1032.

McHugh, M. P., Connolly, D. A. J., Eston, R. G., et al. (1999). The role of passive muscle stiffness in symptoms of exercise-induced muscle damage. *The American journal of sports medicine, 27*(5), 594-599. doi:10.1177/03635465990270050801

McKay, B. R., De Lisio, M., Johnston, A. P. W., et al. (2009). Association of interleukin-6 signalling with the muscle stem cell response following muscle-lengthening contractions in humans. *PLoS One, 4*(6), e6027. doi:10.1371/journal.pone.0006027

McKay, B. R., Toth, K. G., Tarnopolsky, M. A., et al. (2010). Satellite cell number and cell cycle kinetics in response to acute myotrauma in humans: immunohistochemistry versus flow cytometry. *The Journal of Physiology, 588*(17), 3307-3320.

McLennan, I. (1996). Degenerating and regenerating skeletal muscles contain several subpopulations of macrophages with distinct spatial and temporal distributions. *Journal of anatomy, 188*(Pt 1), 17.

McLoughlin, T. J., Mylona, E., Hornberger, T. A., et al. (2003). Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions. *Journal of Applied Physiology*.

McNulty, K. L., Elliott-Sale, K. J., Dolan, E., et al. (2020). The effects of menstrual cycle phase on exercise performance in eumenorrheic women: a systematic review and meta-analysis. *Sports Medicine*. doi:10.1007/s40279-020-01319-3

Meissner, G., Darling, E. & Eveleth, J. (1986). Kinetics of rapid calcium release by sarcoplasmic reticulum. Effects of calcium, magnesium, and adenine nucleotides. *Biochemistry, 25*(1), 236-244.

Melzack, R. (1982). Recent concepts of pain. *Journal of medicine, 13*(3), 147-160.

Mendham, A. E., Donges, C. E., Liberts, E. A., et al. (2011). Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. *Eur J Appl Physiol, 111*(6), 1035-1045. doi:10.1007/s00421-010-1724-z

Mikkelsen, U. R., Langberg, H., Helmark, I. C., et al. (2009). Local NSAID infusion inhibits satellite cell proliferation in human skeletal muscle after eccentric exercise. *Journal of applied physiology, 107*(5), 1600-1611. doi:10.1152/japplphysiol.00707.2009

Miller, A. E., MacDougall, J. D., Tarnopolsky, M. A., et al. (1993). Gender differences in strength and muscle fiber characteristics. *Eur J Appl Physiol Occup Physiol, 66*(3), 254-262. doi:10.1007/bf00235103

Miller, B. F., Hansen, M., Olesen, J. L., et al. (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*(1), E163-E168. doi:10.1152/ajpendo.00300.2005

Minahan, C., Joyce, S., Bulmer, A., et al. (2015_a). The influence of estradiol on muscle damage and leg strength after intense eccentric exercise. *European journal of applied physiology, 115*(7), 1493-1500.

Mitchell, C. J., Churchward-Venne, T. A., West, D. W. D., et al. (2012). Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *Journal of applied physiology, 113*(1), 71-77. doi:10.1152/japplphysiol.00307.2012

Mitchell, C. J., McGregor, R. A., D'Souza, R. F., et al. (2015). Consumption of milk protein or whey protein results in a similar increase in muscle protein synthesis in middle aged men. *Nutrients, 7*(10), 8685-8699. doi:10.3390/nu7105420

Moher, D., Liberati, A., Tetzlaff, J., et al. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS med, 6*(7), e1000097.

Moore, D. R., Atherton, P. J., Rennie, M. J., et al. (2011). Resistance exercise enhances mTOR and MAPK signalling in human muscle over that seen at rest after bolus protein ingestion. *Acta physiologica, 201*(3), 365-372. doi:10.1111/j.1748-1716.2010.02187.x

Moore, D. R., Robinson, M. J., Fry, J. L., et al. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *The American journal of clinical nutrition, 89*(1), 161-168.

Morawetz, D., Blank, C., Koller, A., et al. (2020). Sex-related differences after a single bout of maximal eccentric exercise in response to acute effects: a systematic review and meta-analysis. *The Journal of Strength & Conditioning Research, 34*(9), 2697-2707. doi:10.1519/jsc.0000000000002867

Morgan, D. (1990). New insights into the behavior of muscle during active lengthening. *Biophysical journal, 57*(2), 209-221.

Morgan, D., Claflin, D. & Julian, F. (1996). The effects of repeated active stretches on tension generation and myoplasmic calcium in frog single muscle fibres. *The Journal of Physiology, 497*(3), 665-674.

Moritani, T. (1979). Neural factors versus hypertrophy in the time course of muscle strength gain. *American journal of physical medicine, 58*(3), 115-130.

Morton, R. W., Murphy, K. T., McKellar, S. R., et al. (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*(6), 376-384. doi:10.1136/bjsports-2017-097608

Morton, R. W., Oikawa, S. Y., Wavell, C. G., et al. (2016). Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. *J Appl Physiol (1985), 121*(1), 129-138. doi:10.1152/japplphysiol.00154.2016

Morton, R. W., Sonne, M. W., Farias Zuniga, A., et al. (2019). Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure. *The Journal of Physiology, 597*(17), 4601-4613. doi:10.1113/jp278056

Morton, J. P., Atkinson, G., MacLaren, D. P. M., et al. (2005). Reliability of maximal muscle force and voluntary activation as markers of exercise-induced muscle damage. *European journal of applied physiology, 94*(5), 541-548. doi:10.1007/s00421-005-1373-9

Ms, S. A. B., Waldman, P. H. S., Krings, P. B. M., et al. (2020). Effect of curcumin supplementation on exercise-induced oxidative stress, inflammation, muscle damage,

and muscle soreness. *Journal of Dietary Supplements, 17*(4), 401-414. doi:10.1080/19390211.2019.1604604

Munn, Z., Peters, M. D. J., Stern, C. et al. (2018). Systematic review or scoping review? Guidance for authors when choosing between a systematic or scoping review approach. *BMC Med Res Methodol,* 18(143). doi.org/10.1186/s12874-018-0611-x

Murphy, R. M., Goodman, C. A., McKenna, M. J., et al. (2007). Calpain-3 is autolyzed and hence activated in human skeletal muscle 24 h following a single bout of eccentric exercise. *Journal of Applied Physiology, 103*(3), 926-931. doi:10.1152/japplphysiol.01422.2006

Myllyaho, M. M., Ihalainen, J. K., Hackney, A. C., et al. (2018). Hormonal contraceptive use does not affect strength, endurance, or body composition adaptations to combined strength and endurance training in women. *Journal of Strength and Conditioning Research*. doi:10.1519/jsc.0000000000002713

Naclerio, F., Larumbe-Zabala, E., Cooper, K., et al. (2020). Effects of a multi-ingredient beverage on recovery of contractile properties, performance, and muscle soreness after hard resistance training sessions. *J Strength Cond Res, 34*(7), 1884-1893. doi:10.1519/jsc.0000000000003397

Nana, A., Slater, G. J., Stewart, A. D., et al. (2015). Methodology review: using dual-energy X-ray absorptiometry (DXA) for the assessment of body composition in athletes and active people. *International journal of sport nutrition and exercise metabolism, 25*(2), 198-215.

Newham, D., Jones, D., Ghosh, G., et al. (1988). Muscle fatigue and pain after eccentric contractions at long and short length. *Clinical science, 74*(5), 553-557.

Newham, D., McPhail, G., Mills, K., et al. (1983). Ultrastructural changes after concentric and eccentric contractions of human muscle. *Journal of the neurological sciences, 61*(1), 109-122.

Nguyen, H. X. & Tidball, J. G. (2003). Interactions between neutrophils and macrophages promote macrophage killing of rat muscle cells in vitro. *The Journal of Physiology, 547*(1), 125-132.

Nieman, D., Zwetsloot, K. A., Simonson, A. J., et al. (2020). Effects of whey and pea protein supplementation on post-eccentric exercise muscle damage: a randomized trial. *Nutrients, 12*(8), 2382.

Nogueira, F. R. D., Conceição, M. S., Vechin, F. C., et al. (2013). The effect of eccentric contraction velocity on muscle damage: A review. *Isokinetics and Exercise Science, 21*, 1-9. doi:10.3233/IES-2012-0465

Norikazu, H., Maki, S., Osamu, Y., et al. (2013). Milk peptide intake may decrease muscle damage after eccentric exercise. *International Journal of Sport & Health Science, 11*, 20-28.

Norton, J. P., Clarkson, P. M., Graves, J. E., et al. (1985). Serum creatine kinase activity and body composition in males and females. *Human biology*, 591-598.

Nosaka, K. & Aoki, M. S. (2011). Repeated bout effect: research update and future perspective. *Brazilian Journal of Biomotricity, 5*(1), 5-15.

Nosaka, K. & Clarkson, P. M. (1996_a). Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Medicine and science in sports and exercise, 28*(8), 953-961. doi:10.1097/00005768-199608000-00003

Nosaka, K. & Clarkson, P. M. (1996_b). Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Medicine & Science in Sports & Exercise, 28*(8), 953-961.

Nosaka, K. & Clarkson, P. M. (1996_c). Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *International journal of sports medicine, 17*(02), 120-127.

Nosaka, K. & Clarkson, P. M. (1994). Effect of eccentric exercise on plasma enzyme activities previously elevated by eccentric exercise. *Eur J Appl Physiol Occup Physiol*, *69*(6), 492-497. doi:10.1007/bf00239865

Nosaka, K. Lavender, A., Newton, M., et al. (2003). Muscle damage in resistance training—is muscle damage necessary for strength gain and muscle hypertrophy?. *International Journal of Sport and Health Science, 1*(1), 1-8.

Nosaka, K. & Newton, M. (2002). Difference in the magnitude of muscle damage between maximal and submaximal eccentric loading. *J Strength Cond Res, 16*(2), 202-208.

Nosaka, K., Newton, M. & Sacco, P. (2002_a). Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scand J Med Sci Sports, 12*(6), 337-346. doi:10.1034/j.1600-0838.2002.10178.x

Nosaka, K., Newton, M. & Sacco, P. (2002_b). Muscle damage and soreness after endurance exercise of the elbow flexors. *Medicine and science in sports and exercise, 34*(6), 920-927. doi:10.1097/00005768-200206000-00003

Nosaka, K., Newton, M., Sacco, P., et al. (2005). Partial protection against muscle damage by eccentric actions at short muscle lengths. *Medicine and science in sports and exercise, 37*(5), 746-753. doi:10.1249/01.mss.0000162691.66162.00

Nosaka, K., Newton, M. & Sacco, P. (2005). Attenuation of protective effect against eccentric exercise-induced muscle damage. *Canadian Journal of applied physiology, 30*(5), 529-542.

Nosaka, K., Sacco, P. & Mawatari, K. (2006). Effects of amino acid supplementation on muscle soreness and damage. *Int J Sport Nutr Exerc Metab, 16*(6), 620-635. doi:10.1123/ijsnem.16.6.620

Nosaka, K. & Sakamoto, K. (2001). Effect of elbow joint angle on the magnitude of muscle damage to the elbow flexors. *Medicine and science in sports and exercise, 33*(1), 22-29. doi:10.1097/00005768-200101000-00005

Nosaka, K., Sakamoto, K., Newton, M., et al. (2001_a). How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine & Science in Sports & Exercise, 33*(9), 1490-1495.

Nosaka, K., Sakamoto, K., Newton, M., et al. (2001_b). The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *European journal of applied physiology, 85*(1), 34-40.

Nosaka, K. & Newton, M. (2002). Difference in the magnitude of muscle damage between maximal and submaximal eccentric loading. *The Journal of Strength & Conditioning Research, 16*(2), 202-208.

Novak, L. P. & Tillery, G. W. (1977). Relationship of serum creatine phosphokinase to body composition. *Human biology*, 375-380.

Nygaard, E. (1981). Skeletal muscle fibre characteristics in young women. *Acta Physiologica Scandinavica, 112*(3), 299-304.

O'hagan, F., Sale, D., MacDougall, J., et al. (1995). Response to resistance training in young women and men. *International journal of sports medicine, 16*(05), 314-321.

O'Reilly, C., McKay, B., Phillips, S., et al. (2008). Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 38*(5), 1434-1442.

Ogilvie, R. W., Armstrong, R. B., Baird, K. E., et al. (1988). Lesions in the rat soleus muscle following eccentrically biased exercise. *American journal of anatomy, 182*(4), 335-346.

Orssatto, L. B. R., Moura, B. M., Bezerra, E. S., et al. (2018). Influence of strength training intensity on subsequent recovery in elderly. *Experimental gerontology, 106*, 232-239. doi:10.1016/j.exger.2018.03.011

Ouzzani, M., Hammady, H., Fedorowicz, Z., et al. (2016). Rayyan—a web and mobile app for systematic reviews. Systematic Reviews, *5*(1), 210.

Owen Jr. J. A. (1975). Physiology of the menstrual cycle. *The American journal of clinical nutrition, 28*(4), 333-338. doi:10.1093/ajcn/28.4.333

Owens, D. J., Twist, C., Cobley, J. N., et al. (2019). Exercise-induced muscle damage: what is it, what causes it and what are the nutritional solutions? *European Journal of Sport Science, 19*(1), 71-85. doi:10.1080/17461391.2018.1505957

Oxfeldt, M., Dalgaard, L. B., Farup, J., et al. (2022). Sex hormones and satellite cell regulation in women. *Translational Sports Medicine, 2022,* 9065923. doi:10.1155/2022/9065923

Parry, B. L., LeVeau, B., Mostofi, N., et al. (1997). Temperature circadian rhythms during the menstrual cycle and sleep deprivation in premenstrual dysphoric disorder and normal comparison subjects. *J Biol Rhythms, 12*(1), 34-46. doi:10.1177/074873049701200106

Paschalis, V., Nikolaidis, M. G., Theodorou, A. A., et al. (2013). The effects of eccentric exercise on muscle function and proprioception of individuals being overweight and underweight. *The Journal of Strength & Conditioning Research, 27*(9), 2542-2551. doi:10.1519/JSC.0b013e31827fc9a6

Paschalis, V., Koutedakis, Y., Jamurtas, A. Z., et al. (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*(1), 184-188.

Paschalis, V., Nikolaidis, M., Giakas, G., et al. (2010). Beneficial changes in energy expenditure and lipid profile after eccentric exercise in overweight and lean women. *Scandinavian Journal of Medicine & Science in Sports, 20*, e103-e111. doi:10.1111/j.1600-0838.2009.00920.x

Pasiakos, S. M., Lieberman, H. R., & McLellan, T. M. (2014). Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: a systematic review. *Sports Medicine, 44*(5), 655-670. doi:10.1007/s40279-013-0137-7

Paulsen, G., Egner, I., Drange, M., et al. (2010). A COX-2 inhibitor reduces muscle soreness, but does not influence recovery and adaptation after eccentric exercise. *Scandinavian Journal of Medicine & Science in Sports, 20*(1), e195-e207.

Paulsen, G., Egner, I., Raastad, T., et al. (2013). Inflammatory markers CD11b, CD16, CD66b, CD68, myeloperoxidase and neutrophil elastase in eccentric exercised human skeletal muscles. *Histochemistry and Cell Biology, 139*(5), 691-715.

Paulsen, G., Mikkelsen, U. R., Raastad, T., et al. (2012). Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev, 18*, 42-97.

Pavis, G. F., Jameson, T. S. O., Dirks, M. L., et al. (2021). 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, 320*(2), E291-E305. doi:10.1152/ajpendo.00454.2020

Peake, J. M., Nosaka, K., Muthalib, M., et al. (2006). Systemic inflammatory responses to maximal versus submaximal lengthening contractions of the elbow flexors. *Exerc Immunol Rev, 12*, 72-85.

Pelkman, C. L., Chow, M., Heinbach, R. A., et al. (2001). Short-term effects of a progestational contraceptive drug on food intake, resting energy expenditure, and body weight in young women. *Am J Clin Nutr, 73*(1), 19-26. doi:10.1093/ajcn/73.1.19

Peñailillo, L., Blazevich, A. J. & Nosaka, K. (2015). Muscle fascicle behavior during eccentric cycling and its relation to muscle soreness. *Medicine and science in sports and exercise, 47*(4), 708-717. doi:10.1249/mss.0000000000000473

Pennings, B., Boirie, Y., Senden, J. M., et al. (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*(5), 997-1005. doi:10.3945/ajcn.110.008102

Perdiguero, E., Sousa-Victor, P., Ruiz-Bonilla, V., et al. (2011). p38/MKP-1–regulated AKT coordinates macrophage transitions and resolution of inflammation during tissue repair. *Journal of Cell Biology, 195*(2), 307-322.

Pette, D. (2002). The adaptive potential of skeletal muscle fibers. *Canadian Journal of applied physiology, 27*(4), 423-448.

Phillips, S., Tipton, K. D., Aarsland, A., et al. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *American Journal of Physiology-Endocrinology and Metabolism, 273*(1), E99-E107.

Phillips. S. & Van Loon, L. J. C. (2011). Dietary protein for athletes: From requirements to optimum adaptation. *Journal of Sports Sciences, 29*(sup1), S29-S38. doi:10.1080/02640414.2011.619204

Phillips, S., Tang, J. & Moore, D. (2009). The role of milk- and soy-based protein in support of muscle protein synthesis and muscle protein accretion in young and elderly persons. *Journal of the American College of Nutrition, 28*, 343-354. doi:10.1080/07315724.2009.10718096

Philpott, J. D., Donnelly, C., Walshe, I. H., et al. (2018). Adding fish oil to whey protein, leucine, and carbohydrate over a six-week supplementation period attenuates muscle soreness following eccentric exercise in competitive soccer players. *Int J Sport Nutr Exerc Metab, 28*(1), 26-36. doi:10.1123/ijsnem.2017-0161

Picardo, C. M., Nichols, M., Edelman, A., et al. (2003). Women's knowledge and sources of information on the risks and benefits of oral contraception. *Journal of the American Medical Women's Association (1972), 58*(2), 112-116.

Pildal, J., Hróbjartsson, A., Jørgensen, K., et al. (2007). Impact of allocation concealment on conclusions drawn from meta-analyses of randomized trials. *International Journal of Epidemiology, 36*(4), 847-857. doi:10.1093/ije/dym087

Pinckaers, P. J. M., Kouw, I. W. K., Gorissen, S. H. M., et al. (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*. doi:10.1093/jn/nxac222

Pinckaers, P. J. M., Kouw, I. W. K., Hendriks, F. K., et al. (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*(12), 1832-1842. doi:10.1017/S0007114521000635

Pitkanen, H. T., Nykanen, T., Knuutinen, J., et al. (2003). Free amino acid pool and muscle protein balance after resistance exercise. *Medicine and science in sports and exercise, 35*(5), 784-792. doi:10.1249/01.mss.0000064934.51751.f9

Pizza, F. X. (2009). Comments on Point:Counterpoint: Estrogen and sex do/do not influence post-exercise indexes of muscle damage, inflammation, and repair. *Journal of Applied Physiology, 106*(3), 1016-1020. doi:10.1152/japplphysiol.00004.2009

Pizza, F. X., Cavender, D., Stockard, A., et al. (1999). Anti-inflammatory doses of ibuprofen: effect on neutrophils and exercise-induced muscle injury. *Int J Sports Med, 20*(2), 98-102. doi:10.1055/s-2007-971100

Pizza, F. X., Koh, T. J., McGregor, S. J., et al. (2002). Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *Journal of Applied Physiology, 92*(5), 1873-1878.

Pizza, F. X., Peterson, J. M., Baas, J. H., et al. (2005). Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. *The Journal of Physiology, 562*(3), 899-913.

Poulios, A., Georgakouli, K., Draganidis, D., et al. (2019). Protein-based supplementation to enhance recovery in team sports: what is the evidence? *Journal of sports science & medicine, 18*(3), 523-536.

Pourabbas, M., Bagheri, R., Hooshmand Moghadam, B., et al. (2021). Strategic ingestion of high-protein dairy milk during a resistance training program increases lean mass, strength, and power in trained young males. *Nutrients, 13*(3), 948. doi:10.3390/nu13030948

Prince, F. P., Hikida, R. S. & Hagerman, F. C. (1977). Muscle fiber types in women athletes and non-athletes. *Pflügers Archiv, 371*(1), 161-165.

Procter-Gray, E., Cobb, K. L., Crawford, S. L., et al. (2008). Effect of oral contraceptives on weight and body composition in young female runners. *Medicine and science in sports and exercise, 40*(7), 1205-1212. doi:10.1249/mss.0b013e31816a0df6

Proske, U. & Morgan, D. L. (2001_a). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of Physiology, 537*(Pt 2), 333-345. doi:10.1111/j.1469-7793.2001.00333.x

Proske, U. & Morgan, D. L. (2001_b). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of Physiology, 537*(2), 333-345. doi:10.1111/j.1469-7793.2001.00333.x

Publicover, S., Duncan, C. & Smith, J. (1978). The use of A23187 to demonstrate the role of intracellular calcium in causing ultrastructural damage in mammalian muscle. *Journal of Neuropathology & Experimental Neurology, 37*(5), 544-557.

Pugh, J. K., Faulkner, S. H., Turner, M. C., et al. (2018). Satellite cell response to concurrent resistance exercise and high-intensity interval training in sedentary, overweight/obese, middle-aged individuals. *European journal of applied physiology, 118*(2), 225-238. doi:10.1007/s00421-017-3721-y

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

Radaelli, R., Fleck, S. J., Leite, T., et al. (2015). Dose-response of 1, 3, and 5 sets of resistance exercise on strength, local muscular endurance, and hypertrophy. *The Journal of Strength & Conditioning Research, 29*(5), 1349-1358. doi:10.1519/jsc.0000000000000758

Radák, Z., Pucsok, J., Mecseki, S., et al. (1999). Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. *Free Radical Biology and Medicine, 26*(7), 1059-1063. doi:https://doi.org/10.1016/S0891-5849(98)00309-8

Rafiq, S., Huma, N., Pasha, I., et al. (2016). Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species. *Asian-Australasian journal of animal sciences, 29*(7), 1022-1028. doi:10.5713/ajas.15.0452

Rahimi, M. H., Mohammadi, H., Eshaghi, H., et al. (2018). The effects of beta-hydroxy-beta-methylbutyrate supplementation on recovery following exercise-induced muscle damage: a systematic review and meta-analysis. *Journal of the American College of Nutrition, 37*(7), 640-649. doi:10.1080/07315724.2018.1451789

Rahimi, M. H., Shab-Bidar, S., Mollahosseini, M., et al. (2017). Branched-chain amino acid supplementation and exercise-induced muscle damage in exercise recovery: A meta-analysis of randomized clinical trials. *Nutrition, 42*, 30-36. doi:https://doi.org/10.1016/j.nut.2017.05.005

Rana, S. R., Chleboun, G. S., Gilders, R. M., et al. (2008). Comparison of early phase adaptations for traditional strength and endurance, and low velocity resistance training programs in college-aged women. *The Journal of Strength & Conditioning Research, 22*(1), 119-127. doi:10.1519/JSC.0b013e31815f30e7

Rankin, P., Goldman, L. P., Puglisi, M. J., et al. (2004). Effect of post-exercise supplement consumption on adaptations to resistance training. *Journal of the American College of Nutrition, 23*(4), 322-330. doi:10.1080/07315724.2004.10719375

Rankin, P., Stevenson, E. & Cockburn, E. (2015). The effect of milk on the attenuation of exercise-induced muscle damage in males and females. *Eur J Appl Physiol, 115*(6), 1245-1261. doi:10.1007/s00421-015-3121-0

Raynaud, F., Fernandez, E., Coulis, G., et al. (2005). Calpain 1–titin interactions concentrate calpain 1 in the Z-band edges and in the N2-line region within the skeletal myofibril. *The FEBS Journal, 272*(10), 2578-2590. doi:https://doi.org/10.1111/j.1742-4658.2005.04683.x

Rechichi, C., Dawson, B. & Goodman, C. (2009). Athletic performance and the oral contraceptive. *Int J Sports Physiol Perform, 4*(2), 151-162. doi:10.1123/ijspp.4.2.151

Reis, E., Frick, U. & Schmidtbleicher, D. (1995). Frequency variations of strength training sessions triggered by the phases of the menstrual cycle. *Int J Sports Med, 16*(8), 545-550. doi:10.1055/s-2007-973052

Renault, V., Thorne, L. E., Eriksson, P. O., et al. (2002). Regenerative potential of human skeletal muscle during aging. *Aging cell, 1*(2), 132-139.

Res, P. T., Groen, B., Pennings, B., et al. (2012). Protein ingestion before sleep improves postexercise overnight recovery. *Medicine & Science in Sports & Exercise, 44*(8), 1560-1569.

Rezzi, S., Ginty, F., Beaumont, M., et al. (2009). Body composition precision with the Lunar iDXA. *Journal of Clinical Densitometry, 3*(12), 402.

Ribeiro, A. S., Picoloto, A., Nunes, J. P., et al.. (2020). Effects of different resistance training loads on the muscle quality index in older women. *Journal of Strength and Conditioning Research*. doi:10.1519/jsc.0000000000003667

Richardson, D. L., Duncan, M. J., Jimenez, A., et al. (2018). The perceptual responses to high-velocity, low-load and low-velocity, high-load resistance exercise in older adults. *Journal of Sports Sciences, 36*(14), 1594-1601.

Rickenlund, A., Carlström, K., Ekblom, B. R., et al. (2004). Effects of oral contraceptives on body composition and physical performance in female athletes. *The Journal of Clinical Endocrinology & Metabolism, 89*(9), 4364-4370. doi:10.1210/jc.2003-031334

Rinard, J., Clarkson, P. M., Smith, L. L., et al. (2000). Response of males and females to high-force eccentric exercise. *Journal of Sports Sciences, 18*(4), 229-236. doi:10.1080/026404100364965

Ritti-Dias, R. M., Avelar, A., Salvador, E. P., et al. (2011). Influence of previous experience on resistance training on reliability of one-repetition maximum test. *The Journal of Strength & Conditioning Research, 25*(5), 1418-1422. doi:10.1519/JSC.0b013e3181d67c4b

Roberts, B. M., Nuckols, G. & Krieger, J. W. (2020). Sex differences in resistance training: a systematic review and meta-analysis. *The Journal of Strength & Conditioning Research, 34*(5), 1448-1460. doi:10.1519/jsc.0000000000003521

Rodemann, H. & Goldberg, A. (1982). Arachidonic acid, prostaglandin E2 and F2 alpha influence rates of protein turnover in skeletal and cardiac muscle. *Journal of Biological Chemistry, 257*(4), 1632-1638.

Rodriguez-Sanchez, N. & Galloway, S. D. (2015). Errors in dual energy x-ray absorptiometry estimation of body composition induced by hypohydration. *International journal of sport nutrition and exercise metabolism, 25*(1), 60-68.

Rogers, S. M. & Baker, M. A. (1997). Thermoregulation during exercise in women who are taking oral contraceptives. *Eur J Appl Physiol Occup Physiol, 75*(1), 34-38. doi:10.1007/s004210050123

Rolfe, G. (2006). Validity, trustworthiness and rigour: quality and the idea of qualitative research. *Journal of advanced nursing, 53*(3), 304-310.

Romance, R., Vargas, S., Espinar, S., et al. (2019). Oral contraceptive use does not negatively affect body composition and strength adaptations in trained women. *International journal of sports medicine, 40*(13), 842-849.

Romero-Parra, N., Cupeiro, R., Alfaro-Magallanes, V. M., et al. (2021_a). Exercise-induced muscle damage during the menstrual cycle: a systematic review and meta-analysis. *The Journal of Strength & Conditioning Research, 35*(2), 549-561. doi:10.1519/jsc.0000000000003878

Romero-Parra, N., Rael, B., Alfaro-Magallanes, V. M., et al. (2021_b). The effect of the oral contraceptive cycle phase on exercise-induced muscle damage after eccentric exercise in resistance-trained women. *The Journal of Strength & Conditioning Research, 35*(2), 353-359. doi:10.1519/jsc.0000000000003897

Rosa-Caldwell, M. E. & Greene, N. P. (2019). Muscle metabolism and atrophy: let's talk about sex. *Biology of sex differences, 10*(1), 43. doi:10.1186/s13293-019-0257-3

Rose, C., Edwards, K., Siegler, J., et al. (2017). Whole-body cryotherapy as a recovery technique after exercise: a review of the literature. *International journal of sports medicine, 38 14*, 1049-1060.

Roth, S. M., Gajdosik, R. & Ruby, B. C. (2001). Effects of circulating estradiol on exercise-induced creatine kinase activity. *Journal of Exercise Physiology Online, 4*(2).

Roth, S. M., Martel, G. F., Ivey, F. M., et al. (2000_a). High-volume, heavy-resistance strength training and muscle damage in young and older women. *Journal of Applied Physiology, 88*(3), 1112-1118.

Roth, S. M., Martel, G. F., Ivey, F. M., et al. (2000_b). Skeletal muscle satellite cell populations in healthy young and older men and women. *The Anatomical Record: An Official Publication of the American Association of Anatomists, 260*(4), 351-358.

Roth, S. M., Martel, G. F., Ivey, F. M., et al. (1999). Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training. *Journal of Applied Physiology, 86*(6), 1833-1840.

Rowlands, D. S., Nelson, A. R., Phillips, S. M., et al. (2015). Protein-leucine fed dose effects on muscle protein synthesis after endurance exercise. *Medicine and science in sports and exercise, 47*(3), 547-555. doi:10.1249/mss.0000000000000447

Rusticus, S. A. & Lovato, C. Y. (2014). Impact of sample size and variability on the power and type I error rates of equivalence tests: A simulation study. *Practical Assessment, Research, and Evaluation, 19*(1), 11.

Rutherford, O. M. & Jones, D. A. (1986). The role of learning and coordination in strength training. *Eur J Appl Physiol Occup Physiol, 55*(1), 100-105. doi:10.1007/bf00422902

Sacheck, J. M., Decker, E. A. & Clarkson, P. M. (2000). The effect of diet on vitamin E intake and oxidative stress in response to acute exercise in female athletes. *European journal of applied physiology, 83*(1), 40-46. doi:10.1007/s004210000252

Sakamaki-Sunaga, M., Min, S., Kamemoto, K., et al. (2016). Effects of menstrual phase–dependent resistance training frequency on muscular hypertrophy and strength. *Journal of Strength and Conditioning Research, 30*(6), 1727-1734.

Salomon, G. (1991). Transcending the qualitative-quantitative debate: the analytic and systemic approaches to educational research. *Educational researcher, 20*(6), 10-18.

Saltin, B., Henriksson, J., Nygaard, E., et al. (1977). Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. *Annals of the New York Academy of Sciences, 301*(1), 3-29. doi:10.1111/j.1749-6632.1977.tb38182.x

Salvadori, A., Fanari, P., Ruga, S., et al. (1993). Creatine kinase and creatine kinase-MB isoenzyme during and after exercise testing in normal and obese young people. *Chest, 102*, 1687-1689. doi:10.1378/chest.102.6.1687

Samadi, A., Gaeini, A. A., Kordi, M. R., et al. (2012). Effect of various ratios of carbohydrate-protein supplementation on resistance exercise-induced muscle damage. *J Sports Med Phys Fitness, 52*(2), 151-157.

Sambasivan, R., Yao, R., Kissenpfennig, A., et al. (2011). Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development, 138*(17), 3647-3656. doi:10.1242/dev.067587

Sands, W. A., Wurth, J. J. & Hewit, J. K. (2012). Basics of strength and conditioning manual. *Colorado Springs, CO: National Strength and Conditioning Association*.

Santos, D. A., Dawson, J. A., Matias, C. N., et al. (2014). Reference values for body composition and anthropometric measurements in athletes. *PLoS One, 9*(5), e97846. doi:10.1371/journal.pone.0097846

Santos, D. A., Gobbo, L. A., Matias, C. N., et al. (2013). Body composition in taller individuals using DXA: A validation study for athletic and non-athletic populations. *Journal of Sports Sciences, 31*(4), 405-413. doi:10.1080/02640414.2012.734918

Saracino, P. G., Saylor, H. E., Hanna, B. R., et al. (2020). Effects of pre-sleep whey vs. plant-based protein consumption on muscle recovery following damaging morning exercise. *Nutrients, 12*(7), 1-16. doi:10.3390/nu12072049

Savage, K. J. & Clarkson, P. M. (2002). Oral contraceptive use and exercise-induced muscle damage and recovery. *Contraception, 66*(1), 67-71. doi:https://doi.org/10.1016/S0010-7824(02)00320-7

Saxton, J. M. & Donnelly, A. E. (1995). Light concentric exercise during recovery from exercise-induced muscle damage. *Int J Sports Med, 16*(6), 347-351. doi:10.1055/s-2007-973018

Sayers, S. P. & Clarkson, P. M. (2001). Force recovery after eccentric exercise in males and females. *European journal of applied physiology, 84*(1), 122-126. doi:10.1007/s004210000346

Schilder, A., Hoheisel, U., Magerl, W., et al. (2014). Sensory findings after stimulation of the thoracolumbar fascia with hypertonic saline suggest its contribution to low back pain. *Pain, 155*(2), 222-231. doi:10.1016/j.pain.2013.09.025

Schilder, A., Magerl, W., Hoheisel, U., et al. (2016). Electrical high-frequency stimulation of the human thoracolumbar fascia evokes long-term potentiation-like pain amplification. *Pain, 157*(10), 2309-2317. doi:10.1097/j.pain.0000000000000649

Schoenfeld, B. J. (2010). The mechanisms of muscle hypertrophy and their application to resistance training. *The Journal of Strength & Conditioning Research, 24*(10), 2857-2872. doi:10.1519/JSC.0b013e3181e840f3

Schoenfeld, B. J. (2012). Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? *Journal of Strength and Conditioning Research, 26*(5), 1441-1453. doi:10.1519/JSC.0b013e31824f207e

Schoenfeld, B. J., Contreras, B., Krieger, J., et al. (2019). Resistance training volume enhances muscle hypertrophy but not strength in trained men. *Medicine and science in sports and exercise, 51*(1), 94-103. doi:10.1249/MSS.0000000000001764

Schoenfeld, B. J., Grgic, J., Ogborn, D., et al. (2017_a). Strength and hypertrophy adaptations between low- vs. high-load resistance training: a systematic review and meta-analysis. *Journal of Strength and Conditioning Research, 31*(12), 3508-3523. doi:10.1519/jsc.0000000000002200

Schoenfeld, B. J., Ogborn, D. & Krieger, J. W. (2017_b). Dose-response relationship between weekly resistance training volume and increases in muscle mass: A systematic review and meta-analysis. *Journal of Sports Sciences, 35*(11), 1073-1082. doi:10.1080/02640414.2016.1210197

Schoenfeld, B. J., Ogborn, D. I., Vigotsky, A. D., et al. (2017_c). Hypertrophic effects of concentric vs. eccentric muscle actions: a systematic review and meta-analysis. *The Journal of Strength & Conditioning Research, 31*(9), 2599-2608.

Schoep, M. E., Adang, E. M. M., Maas, J. W. M., et al. (2019). Productivity loss due to menstruation-related symptoms: a nationwide cross-sectional survey among 32 748 women. *BMJ Open, 9*(6), e026186. doi:10.1136/bmjopen-2018-026186

Schubert, M. M., Seay, R. F., Spain, K. K., et al. (2019). Reliability and validity of various laboratory methods of body composition assessment in young adults. *Clinical Physiology and Functional Imaging, 39*(2), 150-159. doi:https://doi.org/10.1111/cpf.12550

Schuenke, M. D., Herman, J. R., Gliders, R. M., et al. (2012). Early-phase muscular adaptations in response to slow-speed versus traditional resistance-training regimens. *Eur J Appl Physiol, 112*(10), 3585-3595. doi:10.1007/s00421-012-2339-3

Schultz, E., Jaryszak, D. L. & Valliere, C. R. (1985). Response of satellite cells to focal skeletal muscle injury. *Muscle Nerve, 8*(3), 217-222. doi:10.1002/mus.880080307

Schulz, E., Chalmers, I. & Altman, D. G. (2002). The landscape and lexicon of blinding in randomized trials. In: American College of Physicians.

Schulz, E. & Grimes, D. A. (2002). Blinding in randomised trials: hiding who got what. *The Lancet, 359*(9307), 696-700. doi:https://doi.org/10.1016/S0140-6736(02)07816-9

Schwane, J. A. & Armstrong, R. B. (1983). Effect of training on skeletal muscle injury from downhill running in rats. *J Appl Physiol Respir Environ Exerc Physiol, 55*(3), 969-975. doi:10.1152/jappl.1983.55.3.969

Scotland, J. (2012). Exploring the philosophical underpinnings of research: Relating ontology and epistemology to the methodology and methods of the scientific, interpretive, and critical research paradigms. *English language teaching, 5*(9), 9-16.

Scott, W., Stevens, J. & Binder–Macleod, S. A. (2001). Human skeletal muscle fiber type classifications. *Physical therapy, 81*(11), 1810-1816. doi:10.1093/ptj/81.11.1810

Seery, S. & Jakeman, P. (2016). A metered intake of milk following exercise and thermal dehydration restores whole-body net fluid balance better than a carbohydrate–electrolyte solution or water in healthy young men. *British Journal of Nutrition, 116*(6), 1013-1021.

Senna, G. W., Willardson, J. M., Scudese, E., et al. (2016). Effect of different interset rest intervals on performance of single and multijoint exercises with near-maximal loads. *The Journal of Strength & Conditioning Research, 30*(3), 710-716. doi:10.1519/jsc.0000000000001142

Sewright, K., Hubal, M., Kearns, A., et al. (2008). Sex differences in response to maximal eccentric exercise. *Medicine & Science in Sports & Exercise, 40*(2), 242-251.

Shaw, G., Lee-Barthel, A., Ross, M. L., et al. (2016). Vitamin C–enriched gelatin supplementation before intermittent activity augments collagen synthesis. *The American journal of clinical nutrition, 105*(1), 136-143. doi:10.3945/ajcn.116.138594

Shepstone, T. N., Tang, J. E., Dallaire, S., et al. (2005). Short-term high- vs. low-velocity isokinetic lengthening training results in greater hypertrophy of the elbow flexors in young men. *Journal of Applied Physiology, 98*(5), 1768-1776. doi:10.1152/japplphysiol.01027.2004

Sherwood, R. A., Lambert, A., Newham, D. J., et al. (1996). The effect of eccentric exercise on serum creatine kinase activity in different ethnic groups. *Annals of clinical biochemistry, 33*(4), 324-329. doi:10.1177/000456329603300407

Sim, M., Dawson, B., Landers, G., et al. (2015). Oral contraception does not alter typical post-exercise interleukin-6 and hepcidin levels in females. *Journal of Science and Medicine in Sport, 18*(1), 8-12. doi:https://doi.org/10.1016/j.jsams.2013.11.008

Simoneau, J. A. & Bouchard, C. (1989). Human variation in skeletal muscle fiber-type proportion and enzyme activities. *American Journal of Physiology-Endocrinology and Metabolism, 257*(4), E567-E572. doi:10.1152/ajpendo.1989.257.4.E567

Simoneau, J. A., Lortie, G., Boulay, M. R., et al. (1985). Skeletal muscle histochemical and biochemical characteristics in sedentary male and female subjects. *Canadian Journal of Physiology and Pharmacology, 63*(1), 30-35. doi:10.1139/y85-005

Singh, M. A. F., Ding, W., Manfredi, T. J., et al. (1999). Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *American Journal of Physiology-*

Endocrinology and Metabolism, 277(1), E135-E143. doi:10.1152/ajpendo.1999.277.1.E135

Singhal, V., Lawson, E. A., Ackerman, K. E., et al. (2014). Irisin levels are lower in young amenorrheic athletes compared with eumenorrheic athletes and non-athletes and are associated with bone density and strength estimates. *PLoS One, 9*(6), e100218. doi:10.1371/journal.pone.0100218

Sipilä, S., Törmäkangas, T., Sillanpää, E., et al. (2020). Muscle and bone mass in middle-aged women: role of menopausal status and physical activity. *Journal of Cachexia, Sarcopenia and Muscle, 11*(3), 698-709. doi:10.1002/jcsm.12547

Solomon, S. J., Kurzer, M. S. & Calloway, D. H. (1982). Menstrual cycle and basal metabolic rate in women. *The American journal of clinical nutrition, 36*(4), 611-616. doi:10.1093/ajcn/36.4.611

Somani, Y. B., Pawelczyk, J. A., De Souza, M. J., et al. (2019). Aging women and their endothelium: probing the relative role of estrogen on vasodilator function. *American journal of physiology. Heart and circulatory physiology, 317*(2), H395-H404. doi:10.1152/ajpheart.00430.2018

Sonnet, C., Lafuste, P., Arnold, L., et al. (2006). Human macrophages rescue myoblasts and myotubes from apoptosis through a set of adhesion molecular systems. *Journal of cell science, 119*(12), 2497-2507.

Sorichter, S., Mair, J., Koller, A., et al. (1997). Skeletal troponin I as a marker of exercise-induced muscle damage. *Journal of Applied Physiology, 83*(4), 1076-1082. doi:10.1152/jappl.1997.83.4.1076

Sorimachi, H., Kimura, S., Kinbara, K., et al. (1996). Structure and physiological functions of ubiquitous and tissue-specific calpain species: Muscle-specific calpain, p94, interacts with connectin/titin. *Advances in Biophysics, 33*, 101-122. doi:https://doi.org/10.1016/0065-227X(96)81667-4

Sowers, M., Zheng, H., Tomey, K., et al. (2007). Changes in body composition in women over six years at midlife: ovarian and chronological aging. *The Journal of Clinical Endocrinology & Metabolism, 92*(3), 895-901. doi:10.1210/jc.2006-1393

Spangenburg, E. E. & Booth, F. W. (2003). Molecular regulation of individual skeletal muscle fibre types. *Acta Physiologica Scandinavica, 178*(4), 413-424. doi:https://doi.org/10.1046/j.1365-201X.2003.01158.x

St. Pierre Schneider, B., Correia, L. A. & Cannon, J. G. (1999). Sex differences in leukocyte invasion in injured murine skeletal muscle. *Research in Nursing & Health, 22*(3), 243-250. doi:https://doi.org/10.1002/(SICI)1098-240X

Staron, R. S., Hagerman, F. C., Hikida, R. S., et al. (2000). Fiber type composition of the vastus lateralis muscle of young men and women. *Journal of histochemistry & cytochemistry, 48*(5), 623-629. doi:10.1177/002215540004800506

Staron, R. S., Hikida, R. S., Murray, T. F., et al. (1992). Assessment of skeletal muscle damage in successive biopsies from strength-trained and untrained men and women. *European Journal of Applied Physiology and Occupational Physiology, 65*(3), 258-264.

Staron, R. S., Karapondo, D. L., Kraemer, W. J., et al. (1994). Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *Journal of Applied Physiology, 76*(3), 1247-1255. doi:10.1152/jappl.1994.76.3.1247

Staron, R. S., Malicky, E., Leonardi, M., et al. (1990). Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *European Journal of Applied Physiology and Occupational Physiology, 60*(1), 71-79.

Stauber, W., Clarkson, P., Fritz, V., et al. (1990). Extracellular matrix disruption and pain after eccentric muscle action. *Journal of Applied Physiology, 69*(3), 868-874.

Stauber, W. T. (1989). Eccentric action of muscles: physiology, injury, and adaptation. *Exercise and sport sciences reviews, 17*(1), 157-186.

Steensberg, A., van Hall, G., Osada, T., et al. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *The Journal of Physiology, 529 Pt 1*(Pt 1), 237-242. doi:10.1111/j.1469-7793.2000.00237.x

Stefanaki, D. G. A., Dzulkarnain, A. & Gray, S. R. (2019). Comparing the effects of low and high load resistance exercise to failure on adaptive responses to resistance exercise in young women. *Journal of Sports Sciences, 37*(12), 1375-1380. doi:10.1080/02640414.2018.1559536

Steward, R. G., Bateman, L. A., Slentz, C., et al. (2016). The impact of short-term depot-medroxyprogesterone acetate treatment on resting metabolic rate. *Contraception, 93*(4), 317-322. doi:10.1016/j.contraception.2016.01.001

Stone, W. J. & Coulter, S. P. (1994). Strength/endurance effects from three resistance training protocols with women. *The Journal of Strength & Conditioning Research, 8*(4), 231-234.

Stožer, A., Vodopivc, P. & Križančić Bombek, L. (2020). Pathophysiology of exercise-induced muscle damage and its structural, functional, metabolic, and clinical consequences. *Physiology Research*, *69*(4), 565-598. doi:10.33549/physiolres.934371.

Stupka, N., Lowther, S., Chorneyko, K., et al. (2000). Gender differences in muscle inflammation after eccentric exercise. *Journal of Applied Physiology, 89*(6), 2325-2332.

Sung, E., Han, A., Hinrichs, T., et al. (2014). Effects of follicular versus luteal phase-based strength training in young women. *Springerplus, 3*, 668. doi:10.1186/2193-1801-3-668

Suresh, L. & Radfar, L. (2004). Pregnancy and lactation. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 97*(6), 672-682. doi:10.1016/j.tripleo.2004.02.002

Swaminathan, R., Ho, C. & Donnan, S. (1988). Body composition and plasma creatine kinase activity. *Annals of clinical biochemistry, 25*(4), 389-391.

Talbot, J. A. & Morgan, D. L. (1996). Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch. *J Muscle Res Cell Motil, 17*(2), 261-268. doi:10.1007/bf00124247

Talbot, J. A. & Morgan, D. L. (1998). The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *J Muscle Res Cell Motil, 19*(3), 237-245. doi:10.1023/a:1005325032106

Tan, B. (1999). Manipulating resistance training program variables to optimize maximum strength in men: a review. *The Journal of Strength & Conditioning Research*, *13*(3), 289-304.

Tang, J. E., Moore, D. R., Kujbida, G. W., et al. (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. *Journal of Applied Physiology*.

Tanimoto, M., Sanada, K., Yamamoto, K., et al. (2008). Effects of whole-body low-intensity resistance training with slow movement and tonic force generation on muscular size and strength in young men. *J Strength Cond Res, 22*(6), 1926-1938. doi:10.1519/JSC.0b013e318185f2b0

Taraborrelli, S. (2015). Physiology, production and action of progesterone. *Acta Obstetricia et Gynecologica Scandinavica, 94*(S161), 8-16. doi:10.1111/aogs.12771

Teixeira, C. F. P., Zamunér, S. R., Zuliani, J. P., et al. (2003). Neutrophils do not contribute to local tissue damage, but play a key role in skeletal muscle regeneration, in mice injected

with Bothrops asper snake venom. *Muscle Nerve, 28*(4), 449-459. doi:10.1002/mus.10453

Tenberg, S., Nosaka, K. & Wilke, J. (2021). Fascia thickness increases following eccentric exercise leading to delayed onset muscle soreness.

Thomas, A., Bunyan, K. & Tiidus, P. M. (2010). Oestrogen receptor-alpha activation augments post-exercise myoblast proliferation. *Acta physiologica, 198*(1), 81-89. doi:https://doi.org/10.1111/j.1748-1716.2009.02033.x

Thompson, H. S., Almarjawi, A., Sculley, D., et al. (2020). The effect of the menstrual cycle and oral contraceptives on acute responses and chronic adaptations to resistance training: a systematic review of the literature. *Sports Medicine, 50*(1), 171-185. doi:10.1007/s40279-019-01219-1

Thompson, H. S., Hyatt, J-P., De Souza, M. J., et al. (1997). The effects of oral contraceptives on delayed onset muscle soreness following exercise. *Contraception, 56*(2), 59-65. doi:https://doi.org/10.1016/S0010-7824(97)00093-0

Tidball, J. G. (2011). Mechanisms of muscle injury, repair, and regeneration. *Comprehensive Physiology, 1*(4), 2029-2062. doi:10.1002/cphy.c100092

Tidball, J. G. & Daniel, T. L. (1986). Elastic energy storage in rigored skeletal muscle cells under physiological loading conditions. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 250*(1), R56-R64.

Tidball, J. G., Dorshkind, K. & Wehling-Henricks, M. (2014). Shared signaling systems in myeloid cell-mediated muscle regeneration. *Development, 141*(6), 1184-1196. doi:10.1242/dev.098285

Tiidus, P. M. (2003). Influence of estrogen on skeletal muscle damage, inflammation, and repair. *Exercise and sport sciences reviews, 31*(1), 40-44.

Tiidus, P. M. (2018). Oestrogen and a Goldilocks zone for post-damage muscle inflammation and repair? *The Journal of Physiology, 596*(19), 4563-4564. doi:10.1113/JP276870

Tiidus, P. M. & Bombardier, E. (1999). Oestrogen attenuates post-exercise myeloperoxidase activity in skeletal muscle of male rats. *Acta Physiologica Scandinavica, 166*(2), 85-90.

Tiidus, P. M. & Enns, D. L. (2009). Point:counterpoint: estrogen and sex do/do not influence post-exercise indexes of muscle damage, inflammation, and repair. *Journal of Applied Physiology, 106*(3), 1010-1012. doi:10.1152/japplphysiol.90848.2008

Tiidus, P. M., Holden, D., Bombardier, E., et al. (2001). Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. *Canadian Journal of Physiology and Pharmacology, 79*(5), 400-406.

Tipton, K. D. (2015). Nutritional support for exercise-induced injuries. *Sports Medicine, 45*(1), 93-104.

Tipton, K. D., Ferrando, A. A., Phillips, S. M., et al. (1999). Postexercise net protein synthesis in human muscle from orally administered amino acids. *American Journal of Physiology-Endocrinology and Metabolism, 276*(4), E628-E634.

Tipton, K. D., Rasmussen, B. B., Miller, S. L., et al. (2001). Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *American Journal of Physiology-Endocrinology and Metabolism, 281*(2), E197-E206.

Tipton, K. D. & Wolfe, R. R. (2001). Exercise, protein metabolism, and muscle growth. *Int J Sport Nutr Exerc Metab, 11*(1), 109-132. doi:10.1123/ijsnem.11.1.109

Tipton, K. D. (2008). Protein for adaptations to exercise training. *European Journal of Sport Science, 8*(2), 107-118.

Tipton, K. D., Hamilton, D. L. & Gallagher, I. J. (2018). Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans. *Sports Medicine, 48*(1), 53-64. doi:10.1007/s40279-017-0845-5

Toombs, R. J., Ducher, G., Shepherd, J. A., et al. (2012). The impact of recent technological advances on the trueness and precision of dxa to assess body composition. *Obesity, 20*(1), 30-39. doi:https://doi.org/10.1038/oby.2011.211

Torgerson, D. J. & Torgerson, C. J. (2003). Avoiding bias in randomised controlled trials in educational research. *British journal of educational studies, 51*(1), 36-45.

Torres, R., Ribeiro, F., Alberto Duarte, J., et al. (2012). Evidence of the physiotherapeutic interventions used currently after exercise-induced muscle damage: Systematic review and meta-analysis. *Physical Therapy in Sport, 13*(2), 101-114. doi:https://doi.org/10.1016/j.ptsp.2011.07.005

Toumi, H. & Best, T. M. (2003). The inflammatory response: friend or enemy for muscle injury? *Br J Sports Med, 37*(4), 284-286. doi:10.1136/bjsm.37.4.284

Trommelen, J., Holwerda, A. M., Kouw, I. W., et al. (2016). Resistance exercise augments postprandial overnight muscle protein synthesis rates. *Medicine and Science in Sports and Exercise, 48*(12), 2517-2525. doi:10.1249/MSS.0000000000001045

Trommelen, J., Kouw, I. W. K., Holwerda, A. M., et al. (2018). Presleep dietary protein-derived amino acids are incorporated in myofibrillar protein during postexercise

overnight recovery. *American Journal of Physiology-Endocrinology and Metabolism, 314*(5), E457-E467. doi:10.1152/ajpendo.00273.2016

Trommelen, J. & van Loon, L. J. C. (2016). Pre-sleep protein ingestion to improve the skeletal muscle adaptive response to exercise training. *Nutrients, 8*(12). doi:10.3390/nu8120763. (Accession No. 27916799)

Tsivitse, S. K., McLoughlin, T. J., Peterson, J. M., et al. (2003). Downhill running in rats: influence on neutrophils, macrophages, and MyoD+ cells in skeletal muscle. *European journal of applied physiology, 90*(5-6), 633-638.

Twist, C. & Eston, R. (2005). The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance. *European journal of applied physiology, 94*(5), 652-658. doi:10.1007/s00421-005-1357-9

Uchida, M. C., Nosaka, K., Ugrinowitsch, C., et al. (2009). Effect of bench press exercise intensity on muscle soreness and inflammatory mediators. *Journal of Sports Sciences, 27*(5), 499-507.

Urso, M. L. (2013). Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment? *Journal of Applied Physiology, 115*(6), 920-928. doi:10.1152/japplphysiol.00036.2013

Váczi, M., Costa, A., Rácz, L., et al. (2009). Effects of consecutive eccentric training at different range of motion on muscle damage and recovery. *Acta Physiol Hung, 96*(4), 459-468. doi:10.1556/APhysiol.96.2009.4.6

Van Thienen, R., D'Hulst, G., Deldicque, L., et al. (2014). Biochemical artifacts in experiments involving repeated biopsies in the same muscle. *Physiol Rep, 2*(5), e00286. doi:10.14814/phy2.286

Van Vliet, S., Beals, J. W., Holwerda, A. M., et al. (2019). Time-dependent regulation of postprandial muscle protein synthesis rates after milk protein ingestion in young men. *Journal of Applied Physiology, 127*(6), 1792-1801.

van Vliet, S., Burd, N. A. & van Loon, L. J. (2015). The skeletal muscle anabolic response to plant- versus animal-based protein consumption. *The Journal of nutrition, 145*(9), 1981-1991. doi:10.3945/jn.114.204305

Varga, T., Mounier, R., Gogolak, P., et al. (2013). Tissue LyC6− macrophages are generated in the absence of circulating LyC6− monocytes and Nur77 in a model of muscle regeneration. *The Journal of Immunology, 191*(11), 5695-5701.

Velders, M., Schleipen, B., Fritzemeier, K. H., et al. (2012). Selective estrogen receptor-β activation stimulates skeletal muscle growth and regeneration. *The FASEB Journal, 26*(5), 1909-1920. doi:https://doi.org/10.1096/fj.11-194779

Verdijk, L. B., Snijders, T., Drost, M., et al. (2014). Satellite cells in human skeletal muscle; from birth to old age. *Age, 36*(2), 545-557.

Verdijk, L. B., van Loon, L., Meijer, K., et al. (2009). One-repetition maximum strength test represents a valid means to assess leg strength in vivo in humans. *Journal of Sports Sciences, 27*(1), 59-68. doi:10.1080/02640410802428089

Viken, H., Reitlo, L. S., Zisko, N., et al. (2019). Predictors of dropout in exercise trials in older adults: the generation 100 study. *Medicine and science in sports and exercise, 51*(1), 49-55.

Vikne, H., Refsnes, P. E., Ekmark, M., et al. (2006). Muscular performance after concentric and eccentric exercise in trained men. *Medicine and science in sports and exercise, 38*(10), 1770-1781.

Vincent, H. & Vincent, K. (1997). The effect of training status on the serum creatine kinase response, soreness and muscle function following resistance exercise. *International journal of sports medicine, 28*(06), 431-437.

Vollestad, N. & Blom, P. (1985). Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiologica Scandinavica, 125*(3), 395-405. doi:https://doi.org/10.1111/j.1748-1716.1985.tb07735.x

Waller, R., Straker, L., O'Sullivan, P., et al. (2015). Reliability of pressure pain threshold testing in healthy pain free young adults. *Scandinavian Journal of Pain, 9*(1), 38-41. doi:doi:10.1016/j.sjpain.2015.05.004

Walters, J. & Baborie, A. (2020). Muscle biopsy: what and why and when? *Practical Neurology, 20*(5), 385-395. doi:10.1136/practneurol-2019-002465

Wang, N., Hikida, R. S., Staron, R. S., et al. (1993). Muscle fiber types of women after resistance training — Quantitative ultrastructure and enzyme activity. *Pflügers Archiv, 424*(5), 494-502. doi:10.1007/BF00374913

Wang, N., Melton, D. W., Porter, L., et al. (2014). Altered macrophage phenotype transition impairs skeletal muscle regeneration. *The American journal of pathology, 184*(4), 1167-1184.

Warren, G. L., Lowe, D. A. & Armstrong, R. B. (1999). Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med, 27*(1), 43-59. doi:10.2165/00007256-199927010-00004

Warren, G. L., Lowe, D. A., Hayes, D. A., et al. (1993). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *The Journal of Physiology*, *468*(1), 487-499. doi:10.1113/jphysiol.1993.sp019783

Warren, G. L., O'Farrell, L., Rogers, K. R., et al. (2006). CK-MM autoantibodies: prevalence, immune complexes, and effect on CK clearance. *Muscle & Nerve, 34*(3), 335-346. doi:https://doi.org/10.1002/mus.20594

Warren, G. L., Lowe, D. A., Hayes, D. A., et al. (1993). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *The Journal of Physiology, 468,* 487-499. doi:10.1113/jphysiol.1993.sp019783

Waskiewicz, A. J., Johnson, J. C., Penn, B., et al. (1999). Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. *Mol Cell Biol, 19*(3), 1871-1880. doi:10.1128/mcb.19.3.1871

Watson, T. A., Callister, R., Taylor, R. D., et al. (2005). Antioxidant restriction and oxidative stress in short-duration exhaustive exercise. *Medicine & Science in Sports & Exercise, 37*(1), 63-71. doi:10.1249/01.Mss.0000150016.46508.A1

Welle, S., Thornton, C., Statt, M., et al. (1994). Postprandial myofibrillar and whole body protein synthesis in young and old human subjects. *American Journal of Physiology-Endocrinology and Metabolism, 267*(4), E599-E604. doi:10.1152/ajpendo.1994.267.4.E599

Wernbom, M., Augustsson, J. & Thomeé, R. (2007). The influence of frequency, intensity, volume and mode of strength training on whole muscle cross-sectional area in humans. *Sports Medicine, 37*(3), 225-264.

West, D. W. D., Abou Sawan, S., Mazzulla, M., et al. (2017). Whey protein supplementation enhances whole body protein metabolism and performance recovery after resistance exercise: a double-blind crossover study. *Nutrients, 9*(7). doi:10.3390/nu9070735

West, D. W. D., Burd, N. A., Churchward-Venne, T. A., et al. (2012). Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *Journal of Applied Physiology, 112*(11), 1805-1813.

West, D. W. D., Miller, W. M., Jeon, S., et al. (2020). The effects of a preconditioning rolling session on subsequent eccentric exercise–induced muscle damage. *The Journal of Strength & Conditioning Research, 34*(8), 2112-2119. doi:10.1519/jsc.0000000000003678

White, J. P., Wilson, J. M., Austin, K. G., et al. (2008). Effect of carbohydrate-protein supplement timing on acute exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition, 5*(1), 1-7. doi:10.1186/1550-2783-5-5

Wiecek, M., Maciejczyk, M., Szymura, J., et al. (2017). Sex differences in oxidative stress after eccentric and concentric exercise. *Redox Report, 22*(6), 478-485. doi:10.1080/13510002.2017.1304195

Wierman, M. E. (2007). Sex steroid effects at target tissues: mechanisms of action. *Advances in Physiology Education, 31*(1), 26-33. doi:10.1152/advan.00086.2006

Wiik, A., Ekman, M., Johansson, O., et al. (2008). Expression of both oestrogen receptor alpha and beta in human skeletal muscle tissue. *Histochemistry and Cell Biology, 131*(2), 181. doi:10.1007/s00418-008-0512-x

Wikström-Frisén, L., Boraxbekk, C. J. & Henriksson-Larsén, K. (2017). Effects on power, strength and lean body mass of menstrual/oral contraceptive cycle based resistance training. *J Sports Med Phys Fitness, 57*(1-2), 43-52. doi:10.23736/s0022-4707.16.05848-5

Wilke, J. & Behringer, M. (2021). Is "delayed onset muscle soreness" a false friend? the potential implication of the fascial connective tissue in post-exercise discomfort. *International Journal of Molecular Sciences, 22*(17), 9482.

Wilkinson, D. J., Bukhari, S. S. I., Phillips, B. E., et al. (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*(6 Pt A), 2011-2021. doi:10.1016/j.clnu.2017.09.008

Wilkinson, D. J., Tarnopolsky, M. A., MacDonald, M. J., et al. (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*(4), 1031-1040.

Williams, P. E. & Goldspink, G. (1973). The effect of immobilization on the longitudinal growth of striated muscle fibres. *J Anat, 116*(Pt 1), 45-55.

Williamson, D., Gallagher, P., Harber, M., et al. (2003). Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. *The Journal of Physiology, 547*(3), 977-987. doi:10.1111/j.1469-7793.2003.00977.x

Willoughby, D. S., VanEnk, C. & Taylor, L. (2003). Effects of concentric and eccentric contractions on exerciseinduced muscle injury, inflammation, and serum il-6. *Journal of Exercise Physiology Online, 6*(4).

Witard, O. C., Garthe, I. & Phillips, S. M. (2019). Dietary protein for training adaptation and body composition manipulation in track and field athletes. *Int J Sport Nutr Exerc Metab, 29*(2), 165-174. doi:10.1123/ijsnem.2018-0267

Witard, O. C., Jackman, S. R., Breen, L., et al. (2014). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr, 99*(1), 86-95. doi:10.3945/ajcn.112.055517

Witard, O. C., Wardle, S. L., Macnaughton, L. S., et al. (2016). Protein considerations for optimising skeletal muscle mass in healthy young and older adults. *Nutrients, 8*(4), 181. doi:10.3390/nu8040181

Wojcik, R. J., Walberg-Rankin, J., Smith, L. L., et al. (2001). Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. *International Journal of Sport Nutrition & Exercise Metabolism, 11*(4), 406-419.

Woledge, R. C., Curtin, N. A. & Homsher, E. (1985). Energetic aspects of muscle contraction. *Monogr Physiol Soc, 41*, 1-357.

Wolfson, R. L. & Sabatini, D. M. (2017). The dawn of the age of amino acid sensors for the mTORC1 pathway. *Cell Metab, 26*(2), 301-309. doi:10.1016/j.cmet.2017.07.001

Wright Jr., K. P. & Badia, P. (1999). Effects of menstrual cycle phase and oral contraceptives on alertness, cognitive performance, and circadian rhythms during sleep deprivation. *Behav Brain Res, 103*(2), 185-194. doi:10.1016/s0166-4328(99)00042-x

Wüst, R. C. I., Morse, C. I., De Haan, A., et al. (2008). Sex differences in contractile properties and fatigue resistance of human skeletal muscle. *Experimental Physiology, 93*(7), 843-850. doi:https://doi.org/10.1113/expphysiol.2007.041764

Yablonka-Reuveni, Z., Rudnicki, M. A., Rivera, A. J., et al. (1999). The transition from proliferation to differentiation is delayed in satellite cells from mice lacking MyoD. *Developmental biology, 210*(2), 440-455.

Yang, Y., Breen, L., Burd, N. A., et al. (2012). Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *British Journal of Nutrition, 108*(10), 1780-1788. doi:10.1017/S0007114511007422

Yin, H., Price, F. & Rudnicki, M. A. (2013). Satellite cells and the muscle stem cell niche. *Physiological reviews, 93*(1), 23-67. doi:10.1152/physrev.00043.2011

Yoon, E-J. & Kim, J. (2020). Effect of body fat percentage on muscle damage induced by high-intensity eccentric exercise. *International Journal of Environmental Research and Public Health, 17*, 3476. doi:10.3390/ijerph17103476

Yu, J-G., Carlsson, L. & 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*(3), 219-227. doi:10.1007/s00418-004-0625-9

Zainuddin, Z., Newton, M., Sacco, P., et al. (2005). Effects of massage on delayed-onset muscle soreness, swelling, and recovery of muscle function. *Journal of Athletic Training, 40*(3), 174-180.

Zeller, B. L., McCrory, J. L., Kibler, W. B., et al. (2003). Differences in kinematics and electromyographic activity between men and women during the single-legged squat. *Am J Sports Med, 31*(3), 449-456. doi:10.1177/03635465030310032101

Zhang, S., Osumi, H., Uchizawa, A., et al. (2020). Changes in sleeping energy metabolism and thermoregulation during menstrual cycle. *Physiological Reports, 8*(2), e14353-e14353. doi:10.14814/phy2.14353

Zhang, S., Xiao, Z., Qu, C., et al.. (2014). CD8 T cells are involved in skeletal muscle regeneration through facilitating MCP-1 secretion and Gr1high macrophage infiltration. *The Journal of Immunology, 193*(10), 5149-5160.

Zhang, S., Zhao, T., Zhang, Y., et al. (2020). Avenanthramide supplementation reduces eccentric exercise-induced inflammation in young men and women. *Journal of the International Society of Sports Nutrition, 17*(1), 41. doi:10.1186/s12970-020-00368-3

Zhou, S. (2000). Chronic neural adaptations to unilateral exercise: mechanisms of cross education. *Exerc Sport Sci Rev, 28*(4), 177-184.

APPENDICES

Appendix 1. *General methodology*

Primary Data

Study Design

A parallel group, single-blind, RCT was conducted to investigate sex-based responses to EIMD with milk protein ingestion. Four independent groups were included: female protein (Female-PRO), female control (Female-CON), male protein (Male-PRO), and male control (Male-CON). A parallel group design was appropriate for this trial because the participants had no previous experience in resistance exercise and so some degree of muscle damage should be expected, irrespective of dietary condition. A crossover design is less appropriate, as exercise training can attenuate EIMD (Balnave & Thompson, 1993) owing to repeated bout effects (Hough, 1902) and perhaps confound the impact of protein supplementation. Further, a crossover trial, while requiring fewer participants, would have extended the study duration, as a second washout period of at least 2 weeks would have been needed between the dietary conditions. This additional time for participants may have reduced their willingness to volunteer.

Following study enrolment and baseline testing, participants were equally and randomly assigned to their dietary condition (milk-protein or control) to limit researcher bias. Bias can arise if group allocation is not randomised, as the researcher may intentionally or subconsciously select individuals with certain attributes or desirable characteristics for a chosen treatment group, which could result in an unrepresentative sample of the population (Torgerson & Torgerson, 2003). Therefore, randomisation allows for balanced participant characteristics between groups, thus reducing the influence of extraneous variables on the study outcomes (Hariton & Locascio, 2018). To achieve randomisation, participants drew a folded piece of paper labelled ‘A’ or ‘B’ from an opaque envelope. Separate envelopes were used for male and female participants to ensure an equal balance of sexes across groups (4 males and 8 females in each group).

Participants were unaware of their treatment condition (blinded) to reduce participant bias. For instance, bias may arise if the participant believed they were receiving a supplement that they thought should reduce their symptoms of muscle soreness and consequently reported lower muscle soreness ratings to fit this ‘expectation’ (Schulz et al., 2002). The trial was single-

blinded, whereby only the participant was unaware of their treatment condition, as opposed to double-blinded, whereby both the participant and researcher were unaware of the treatment condition (Schulz & Grimes, 2002), due to logistical reasons. Specifically, the yoghurt had to be frequently purchased, prepared, and delivered to the participant, often out-of-hours, by the researcher and so it was not feasible for this to be done by someone outside of the research team. Then, to avoid researcher bias, i.e. the intentional manipulation of data by, for example, using different verbal or body language toward one group of participants than the other (Torgerson & Torgerson, 2003), the researcher ensured that conditions were matched across all participants. For example, by providing the same verbal encouragement during the muscle damage exercise protocol and phrasing questions in the same manner. To achieve single-blinding, participants were provided with their allocated yoghurt in clear, plastic pots labelled 'A' or 'B'. The yoghurts were similar in appearance, taste, and texture, and the participants were not informed of the two yoghurt types being investigated.

Study Sample and Sampling Strategy

A statistical power analysis was conducted using G*Power 3.1 to determine the study sample size. The power calculation was based on a study by Cockburn et al. (2008), as this study also employed a parallel group randomised design in which participants performed a single bout of high-intensity leg flexion exercise and consumed milk protein or a carbohydrate-based beverage post-exercise. This study reported a significant difference in the change in isokinetic MVC from baseline to 48 h post-exercise between the milk protein (semi-skimmed milk, 17 g protein per dose) and carbohydrate groups of 25%. The relative change in isokinetic MVC was the chosen outcome for the power calculation because the post-exercise change in MVC is considered the most valid indirect marker of EIMD (Damas et al., 2016$_a$). There are some differences between the present study (*chapter 6*) and that of Cockburn et al. (2008), which may impact the ability to detect significant differences within the sample population in response to the intervention. Specifically, Cockburn et al. studied young, trained males, while the present study recruited young, untrained males and females, and training status can impact EIMD responses (Balnave & Thompson, 1993; Ertel et al., 2020; Vincent & Vincent, 1997). Further, the exercise protocol used by Cockburn et al. involved a prescribed volume of unilateral knee flexions, whereas the present study involved an undefined volume (repetitions to failure) of bilateral leg flexions and extensions. Cockburn et al. supplemented participants with two doses of fluid cow's milk post-exercise, while the present study supplemented participants with a total of 17 doses of milk protein sourced from dairy yoghurt. Finally, the

tests of muscle function varied, with the present study measuring the change in 1RM (isotonic contraction) and Cockburn et al. assessing the change in isokinetic MVC. However, there was not a more comparable study to the present that reported significant between-group differences in EIMD markers and so the study by Cockburn et al. (2008) was the most appropriate to use for the sample size calculation. The power calculation revealed that 5 participants per group (20 total) were required to have 80% power to detect a significant difference between groups when using a dependent *t* test with a 0.05 two-sided significance level. Therefore, 24 participants were recruited to allow for 20% drop-out rate.

Twenty-four healthy, adult volunteers (16 female, 8 male) were recruited to participate in this trial via a convenience sampling strategy. Prospective participants were recruited from the Durham University student and staff population via the 'dialogue signposts' email circulation as well as word of mouth. Although an easy and time-efficient strategy, a disadvantage of convenience sampling is a reduced likelihood of obtaining a representative sample of the population compared to probability-type sampling methods, such as random sampling (Landreneau & Creek, 2009). However, given the target population were young adults, the obtained sample is likely fairly representative of this population. Participants were stratified by sex because males and females can experience varied responses to EIMD (Kerksick et al., 2008; MacIntyre et al., 2000; Minahan et al., 2015; Sewright et al., 2008; Stupka et al., 2000) and it was an aim of the study to examine sex differences in EIMD and so equal sex representation across supplement groups was necessary. Interested participants were provided with the participant information (*appendix 2*) and privacy notice (*appendix 3*) documents, and a link to a Microsoft Forms pre-screening eligibility criteria questionnaire (*appendix 4*). The eligibility criteria for inclusion are outlined in *Table A1*.

Table A1. Participant eligibility criteria for inclusion in the randomised controlled trial.

Inclusion	Justification
❖ Males or females	To allow for sex comparison
❖ Aged 18–35 years	Age-based response to EIMD; less likely to have co-morbidities or take medications that may impact EIMD compared to older adults; ease of sampling from university population
❖ Non-obese (BMI ≥18.5 and <30 kg•m^{-2})	BMI-based response to EIMD (Kim & So, 2018); reflective of general health and reduced co-morbidity risk
❖ Untrained in resistance exercise (have not performed resistance exercise in previous 6 months)	Untrained individuals are more likely to experience EIMD response; wanted to target and make outcomes applicable to wider general population, not just athletes (aim is to increase compliance to exercise in novices)
❖ Free from chronic disease, acute illness, and musculoskeletal injury	To not affect ability to exercise or EIMD response; for outcomes to be applied to healthy populations
❖ Able to consume dairy products (not lactose intolerant or do not follow a dairy-free diet)	Study may involve the consumption of milk protein
❖ Do not frequently (twice per week for previous 1-month period) engage in massage, cryotherapy, or acupuncture, or consume performance-enhancing supplements, or non-steroidal anti-inflammatory drugs	May impact response to EIMD
❖ (Females only) Naturally menstruating for previous 12 months (or absence of menstruation due only to hormonal contraceptive use)	Indicative of overall female health and hormone regularity; sex steroid hormones may impact EIMD response (Clarkson & Sayers, 1999)
❖ (Females only) Not pregnant or breast-feeding	Radiation risk with DXA

Ethical Considerations and Risk Assessment

This study received ethical approval from the Department of Sport and Exercise Sciences Research Ethics Committee at Durham University and the Tyne and Wear South NHS Research Ethics Committee. Written informed consent (*appendix 5*) was obtained prior to commencement of any study procedures. A key ethical consideration was the maintenance of participant privacy, confidentiality, and anonymity. To ensure this, participants were allocated an identification number (01–25) at the point of study enrolment, which was used on all

subsequent data collection materials. Data were stored on a password-protected personal laptop and paper data collection sheets were locked within the Human Performance Laboratory, accessible only to the research team. Any identifiable data were either anonymised or destroyed upon study completion and all data included in this thesis and any published materials are unidentifiable.

There were several risks to the participant associated with this research, which were addressed through appropriate risk assessments and mitigation strategies. A key risk arose from the collection of venous blood samples, which carried a risk of infection, discomfort, and minor bruising. Blood collection was conducted by a trained phlebotomist under sterile conditions. There was a risk of musculoskeletal injury during resistance exercise, and therefore a general health and readiness to exercise questionnaire (*appendix 6*) was completed by participants via Microsoft Forms prior to exercise. Participants were shown the correct lifting technique, performed a warm-up, and were supervised at all times. The assessment of body composition with DXA exposes the participant and researcher to a small dose of ionising radiation (effective dose per scan ≈2 μSv). This dose is considered safe and is the equivalent of a few days of natural background radiation in the UK. The researcher wore a radiation detection badge to monitor their total exposure during the study period.

In light of Covid-19, the university adhered to the latest government guidance to reduce the spread of infection. The minimum required number of people were present during laboratory testing (usually participant, researcher, and supervisor); a 2 m distance was maintained when close-contact was not needed; a face covering was worn by both the researcher and participant, except during exercise; all surfaces including the exercise equipment were cleaned before and after use with the correct cleaning solution; frequent hand-washing was performed; and if either a participant or the researcher developed Covid-19 symptoms or received a positive test result they were instructed not to attend the laboratory and to self-isolate in line with government guidance.

Study Procedures and Materials

All study procedures took place in the Human Performance Laboratory, Truscott Imaging Suite, and The Fitness Centre located within the Graham Sports Centre at Maiden Castle, Durham University. A schematic overview of the study procedures is presented in *Figure A1*. The study measured the impact of resistance exercise with milk-protein or control supplement ingestion (independent variable) on 6 dependent variables: maximal leg strength; limb

circumference; ROM; serum [CK]; muscle soreness VRS; and muscle soreness PPT. These variables (excluding maximal strength) were assessed immediately pre, immediately post, and 24, 48, 72, and 168 h post-exercise. Maximal leg strength was assessed with a 1RM test at baseline (-28 and -25 d) and at +72 and +168 h. The baseline 1RM test also served to determine the exercise load (80% of 1RM) for the muscle-damaging exercise protocol. The 1RM test was not conducted during post-48 h to reflect habitual exercise practices, in which a couple of days rest would succeed an exercise bout. Body composition was determined at -25 d for participant descriptive characteristics and to examine its relationship to EIMD (*chapter 7*). A minimum 3-week washout period was included after baseline testing to reduce repeated bout effects on EIMD (Hough, 1902) and to control MC phase. For female participants, all variables were assessed during the self-reported late-follicular phase of the MC to control for sex steroid hormone status within and between participants. Females provided information on the expected onset and duration of their menses and testing sessions were scheduled as close as feasible following the last day of menses to predict the late-follicular phase.

The participants were provided with 17 doses of their allocated yoghurt over the study duration. As instructed, these were consumed as 4 doses per day at ~3–4 h intervals for 4 consecutive days, with the first dose being consumed alongside breakfast prior to the resistance exercise protocol. The final dose was consumed with breakfast at +168 h. Participants were asked to consume a standardised breakfast (2 slices of bread/toast with jam/honey etc., or a bowl of low-sugar cereal/porridge with milk; 238 kcal, 8 g protein, 45 g carbohydrate, 3 g fat) on the morning of testing at +0–72 and +168 h to ensure comparable energy and macronutrient intakes between groups. Participants were asked to avoid consumption of supplemental protein sources (e.g. shakes, bars, gels), though otherwise continued their habitual diet. Habitual diet and activity were self-recorded for 3 days during the 3-week washout period using a template and example for guidance (*appendix 7*). Dietary intake was assessed using the 24 h recall method at +0–72 and +168 h to ensure no unexpected dietary changes had occurred during the study period and to test for any differences between groups. The 24 h dietary recall method is time-efficient, has low participant burden, and generally does not alter habitual intake given it is completed retrospectively (Castell et al., 2015); however, this method has moderate validity relative to the observed intake method (Karvetti & Knuts, 1985).

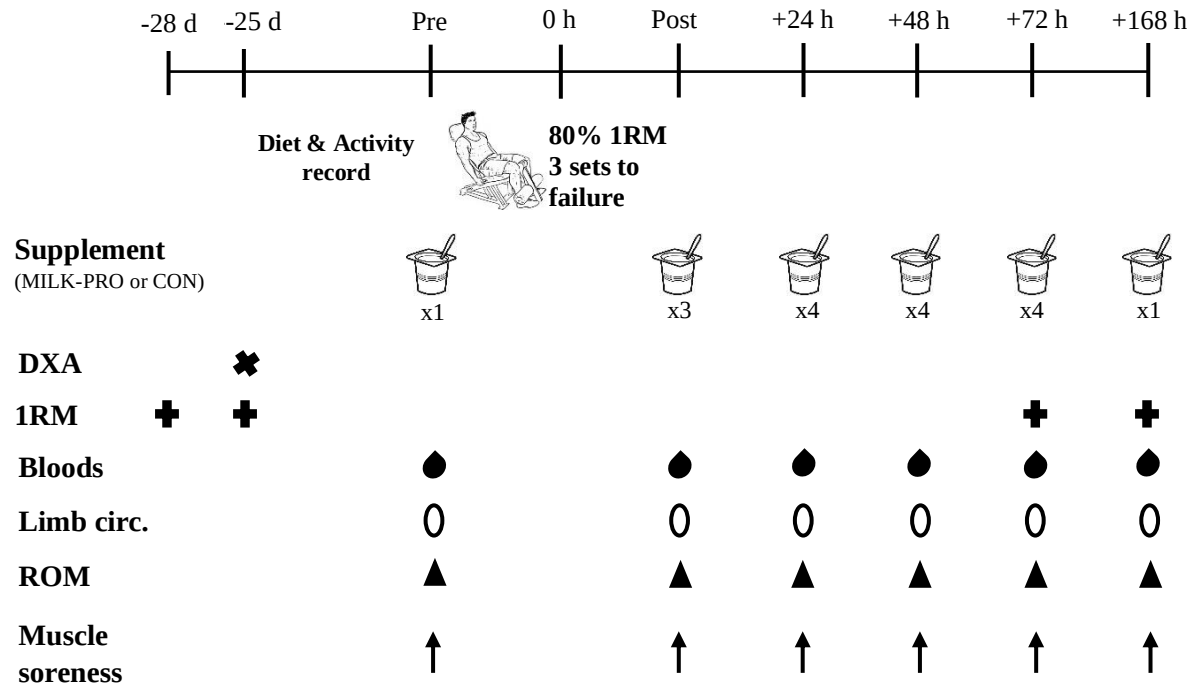


Figure A1. Study design.

Note: DXA = dual-energy X-ray absorptiometry; 1RM = one repetition maximum; limb circ. = limb circumference; ROM = range of motion. MILK-PRO = milk-protein supplement (milk-based yoghurt) providing 20 g of protein; CON = control supplement (isoenergetic oat-based yoghurt).

Dietary Conditions

The milk-protein group consumed 208 g of Arla Skyr strawberry yoghurt (providing 20 g of protein and 152 kcal). Yoghurt was chosen as the source of milk-protein, as opposed to an isolated protein source such as whey, to promote a food-first approach (Burd et al., 2019; Close et al., 2022). Protein obtained from whole-food sources provides other essential nutrients (e.g. carbohydrates, fats, vitamins, and minerals) which are often low in isolated protein sources. Also, milk-based yoghurt offers a complete amino acid profile and is a common, affordable, and easily accessibly dietary staple. Current recommendations for dietary protein as an exercise recovery strategy are to consume ~20 g of high-quality protein every ~3 h peri-exercise (Areta et al., 2013; Moore et al., 2009). These recommendations are based on the ingestion frequency and dosage of protein needed to maximally stimulate muscle protein synthesis rates for muscle repair and growth. Therefore, it was pragmatic to assess whether this protein feeding protocol is beneficial for EIMD.

The control group consumed 150 g of Oatly strawberry Oatgurt (providing 2.1 g of protein and 152 kcal). Oat-based yoghurt was chosen as the control product as it was low in protein while

being similar in appearance, texture, and taste to the milk-protein yoghurt, which allowed for participant blinding. A lesser quantity of the control yoghurt was used for it to be energy-matched with the milk-protein yoghurt. This ensured that any between-group differences in the study outcomes were due to the protein content and not the energy content of the yoghurts. However, the control yoghurt was higher in carbohydrate and fat compared to the milk-protein yoghurt.

Body Composition and Anthropometric Assessment

Body composition was assessed using DXA (GE Lunar iDXA, GE Healthcare, Madison, WI) (*Figure A2.a*) with enCORE software to determine total-body lean mass, fat mass, BF%, and BMC, as well as regional composition of the legs (i.e. the exercised muscle group). Prior to the first scan of each day, a quality assessment was performed to ensure the DXA machine was correctly calibrated.

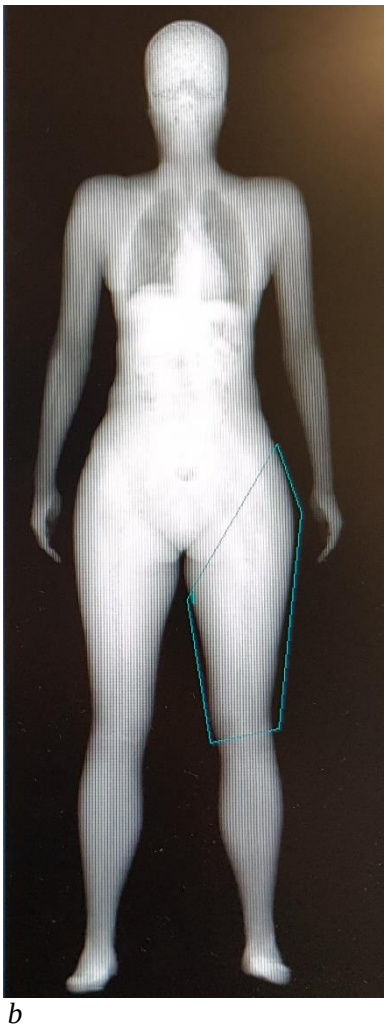
Stature was measured to the nearest mm using a stadiometer and body mass was measured to the nearest 100 g using digital weighing scales (Seca Weighting and Measuring Systems, Birmingham, UK). Participants were positioned centrally and supine on the DXA scan bed with hands in a mid-prone position and head in the frankfort plane. Scan mode (standard = 153 $mm \bullet s^{-1}$; thick = 88 $mm \bullet s^{-1}$) was automatically determined based on estimation of the participant's body thickness (cm) from stature and body mass data (16–25 cm thickness = standard mode; >25 cm thickness = thick mode). Two total-body scans were conducted in succession, with repositioning, to enable calculation of precision error, which has not yet been determined within a population of untrained males and females using this particular model and manufacturer of DXA. The best practice procedures for DXA measurement (Hind et al., 2018; 2022; Nana et al., 2015) (*appendix 8*) were followed to minimise biological variation:

- Fast overnight or for at least 5 h before the scan
- Consume 500 mL of water 3 h before the scan
- Abstain from moderate-vigorous exercise in the 12 h before the scan
- Avoid caffeine consumption for 5 h before the scan
- Avoid alcohol consumption for 24 h before the scan
- Void bladder immediately before the scan
- During the scan, wear light weight, close-fitting clothing that is free from metal, underwire, plastic, or reflective strips, e.g. shorts and t-shirt

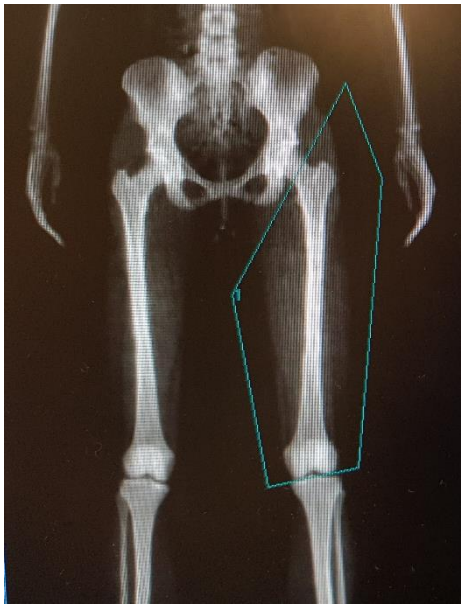
DXA was the chosen method as it is considered the gold-standard method for the assessment of body composition and enables the analysis of regional body composition, which cannot be accurately done using other methods of body composition assessment, e.g. skinfold thickness. iDXA scans are fast (~6.5 min per standard scan), require minimal participant effort, and are safe (~2 μSv effective dose per scan). The iDXA method has low precision error (0.4–0.5%, 0.7–1.0%, and 0.6–0.9% CV) for the assessment of lean mass, fat mass, and percent body fat, respectively among male and female populations of varied training status (Hind et al., 2011; Rezzi et al., 2009; Toombs et al., 2012).

Custom analysis was performed using the enCORE software to manually select the region of interest. The segmental lines were placed at the lower and upper ends of the trochanterion-tibiale lateral site as per *Figure A2.b and A2.c* to encompass the quadricep and hamstring muscles (i.e. exercised muscles) and surrounding fat tissue. Manually selecting regions of interest for lower extremities is highly reliable (r = 0.95–0.98) (Lohman et al., 2009).

a



b



c

Figure A2.a. Lunar iDXA (GE Healthcare, Madison, WI) narrow fan beam machine used for body composition analysis; b. and c. DXA scan output showing the region of interest for the analysis of leg composition.

Muscle Damage Exercise Protocol

The resistance exercise protocol designed to induce muscle damage consisted of leg extension and leg curl exercises performed on a dual plate-loaded machine (Versa leg extension/leg curl, Matrix, Wisconsin, USA) (*Figure A3*). A warm-up set of 10 repetitions was performed with leg extension exercise at 50% of the participants' pre-determined 1RM. Next, 3 working sets at 80% 1RM were performed to volitional failure (when a full repetition could not be performed with correct form), with 2 min inter-set rest. The velocity of movement was 1 sec for the

concentric phase and 2 sec for the eccentric phase. After 5 min rest, this protocol was repeated with leg curl exercise.

Due to the participants being unfamiliar with resistance exercise, machine-based exercise was chosen as this better enables correct form and reduces injury risk compared to free-weight exercise. The plate-loaded machine also allowed for easy and time-efficient incremental (2.3 kg) load increases. A leg-based exercise protocol was performed to mimic a traditional split routine (e.g. leg-day, back-day etc.). A whole-body exercise protocol would have required 1RM testing for multiple exercises, which would have been too time-consuming and fatiguing. For untrained participants, arm-based exercise is less suitable, as maximal arm strength may be rather low and therefore achieving a load of 80% 1RM would be difficult with the minimum increments of 2.3 kg. Also, it is likely that a very low number of repetitions would be completed and may not induce muscle damage.

Many experimental EIMD protocols involve eccentric-only muscle contractions, as these induce the greatest damage (Armstrong et al., 1991; Ebbeling & Clarkson, 1989). However, it was not the goal of this study to maximise damage, but rather use a realistic exercise protocol, including both eccentric and concentric muscle actions. To further mimic real-life training, 3 high-intensity working sets were performed, as opposed to the extreme total work often performed in experimental scenarios (e.g. 100–300 maximal repetitions (Buckley et al., 2010; Burnley et al., 2010; Dale et al., 2015; Draganidis et al., 2017; Farup et al., 2014; Ives et al., 2017; Wojcik et al., 2001)).

Figure A3. Dual plate-loaded leg extension/curl resistance exercise machine used to perform the muscle damage exercise protocol and one repetition maximum test.

EIMD Measures

Maximal Leg Strength

Maximal leg strength was assessed with a 1RM test conducted with leg extension and leg curl exercises, as per the protocol previously described (Baechle & Earle, 2008). The post-exercise change in muscle strength is deemed to best reflect the degree of EIMD and its return to pre-exercise values is often used to mark muscle recovery (Damas et al., 2016_a). Muscle strength is typically measured as a maximal isometric or isokinetic voluntary contraction using dynamometry. However, due to equipment availability, a 1RM test was conducted, which is commonly used (Barroso et al., 2011; Draganidis et al., 2013; Naclerio et al., 2020) and is highly correlated with isometric and isokinetic peak torque using dynamometry for leg extension (r = 0.78 to 0.88) (Verdijk et al., 2009). The 1RM test has good to excellent test-retest reliability (median ICC = 0.97; CV = 4.2%), independent of the sex, age, and training experience of the population (Grgic et al., 2020).

Limb Circumference

The mid, lower-, and upper- quartile points of the trochanterion-tibiale lateral site were measured using an anthropometric segmometer (*Figure A4.a*) with the participant seated on a box and their knee joint at a 90° angle (*Figure A4.b*). These three sites were marked as shown in *Figure A4.c* for future reference. Limb circumference of the dominant leg was then measured using a standard anthropometric measuring tape (*Figure A4.d*) at the three marked sites with the participant in a standing position.

A change in limb circumference suggests muscle swelling, due to increased extracellular fluid, and indicates an inflammatory response. Ultrasound or magnetic resonance imaging can be used to directly measure muscle cross-sectional area and thickness, though standard anthropometric tape was more convenient and readily available. Three sites along the leg were chosen as swelling may differ across regions of the limb depending on the local site of damage.

Range of Motion

The ROM of the knee joint of the dominant leg was calculated as the difference between the relaxed and flexed knee joint angle, as measured using a standard goniometer (*Figure A4.e*) with the participant in a supine position on the phlebotomy bed. Assessing joint ROM is used to indicate flexibility, which may be reduced following EIMD, due to muscle swelling, soreness, and stiffness.

Muscle Soreness

Two methods were used to assess muscle soreness, as responses may differ upon active muscle tension and muscle palpation. To assess active muscle soreness, participants rated their muscle soreness using a VRS ranging from '0 – not sore at all' to '10 – extremely sore' (Burnley et al., 2010; Nosaka et al., 2006) (*appendix 9*) while performing a standard bodyweight squat, which has been done previously (Asadi et al., 2017; Bird et al., 2013; Twist & Eston, 2005). Participants rated separately the soreness in their quadricep, hamstring, glute, and calf muscles after the performance of a single squat (i.e. 4 squats in total). VRSs are a popular pain rating scale for use in clinical trials due to their ease of use, high compliance rates, responsiveness, and good application (Hjermstad et al., 2011). VRSs have demonstrated high test-retest reliability (intraclass correlation coefficient (ICC) [95% CI] = 0.95 [0.93–0.96]) for the assessment of subjective pain and have been validated against both VASs (Pearson's correlation coefficient, $r = 0.941$) and verbal rating scales ($r = 0.925$). VRSs have a minimum detectable change value of 1.33 (Alghadir et al., 2018).

Muscle soreness also was assessed with the PPT test using a computerised pressure algometer (Medoc, AlgoMed, Ramat Yishai, Israel) (*Figure A4.f*) with the participant in a supine position on the phlebotomy bed (*Figure A4.g*). The 1 cm^2 probe head of the algometer was placed at the mid, lower-, and upper- quartile points of the trochanterion-tibiale lateral site and increasing pressure was applied until the participant indicated pain. The PPT test has high test-retest reliability (ICC = 0.96–0.98) for the assessment of leg muscle pain among a comparable population to that used in the current study (Waller et al., 2015) and the use of pressure algometry has been validated against force plate readings ($r = 0.990–0.999$) (Kinser et al., 2009).

Serum Creatine Kinase Concentration

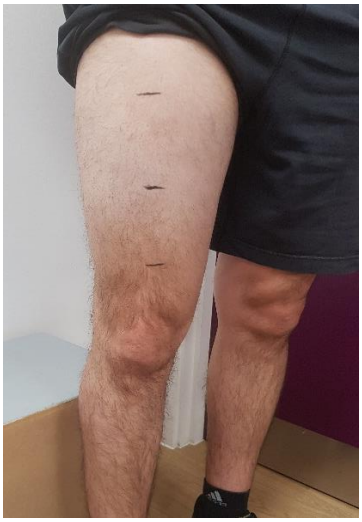
Blood samples were collected from an antecubital vein of the forearm using standard venepuncture techniques with a 21 g vacutainer safety needle and holder into three 10 mL reagent-free vacutainers (*Figure A4.h)*. Samples were left at room temperature for 30 min before being either immediately centrifuged at 4 °C with 1100 g force for 15 min or stored on ice for up to 2 h before centrifuging. Serum samples were transferred into 1.5 mL microcentrifuge tubes and stored at -80 °C until analysis of creatine kinase concentration.

Creatine kinase is an intramuscular enzyme protein and its concentration within systemic circulation indicates myofibre membrane permeability, which may be altered following EIMD.

Venous sampling enabled the collection of sufficient blood volumes for later analysis; 3 ×10 mL vacutainers of whole-blood were collected to allow for 4 × 1.5 mL microcentrifuge tubes to be filled with serum. This amount accounted for any spillages, lost samples, incompletely filled vacutainers, spare serum samples for future analysis, and the analysis of [CK].

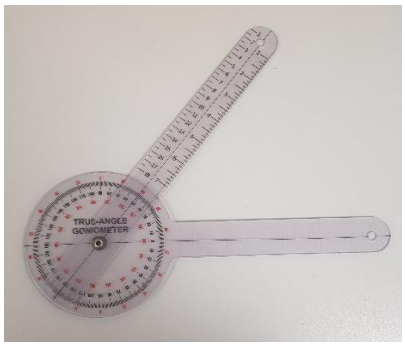
a

b



c

d



e

f

g

h

Figures A4.a–h. Materials used for the assessment of exercise-induced muscle damage: a. segmometer, b. seating positioning during measurement of the trochanterion-tibiale lateral site, c. marked points at the mid, lower-, and upper- quartiles of the trochanterion-tibiale lateral site, d. anthropometric tape, e. goniometer, f. pressure algometer, g. phlebotomy bed, h. 10 mL reagent-free vacutainer (left) and 21 g vacutainer safety needle and holder (right).

Sample and Statistical Analyses

Serum samples were defrosted thoroughly, then [CK] was measured in triplicate to increase reliability of the measure and to allow for any outlying values or errors (e.g. addition of an incorrect reagent). A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abcam plc, Cambridge, UK) was used, which provided the required reagents and a 96-well plate to which the serum samples were added. Serum samples were diluted by a factor of 200 to ensure [CK] could be detected against the standard curve. The sample optical density was measured using a microplate reader set at a wavelength of 450 nm and these values were plotted against the standard curve to determine [CK] ($pg \bullet mL^{-1}$). Abcam report the inter- and intra- assay precision of this ELISA test to be 2.3% and 3.6%, respectively. Out of the five completed tests, two tests (analysing 27 samples each) produced abnormally low [CK] and were deemed void – the reason for this error is unknown. Due to a limited number of assay kits because of financial constraints, not all of these samples could be re-analysed and so the decision was made to re-analyse the samples collected at pre, +48 and +72 h in attempt to capture baseline and peak [CK]. For valid samples, the mean of the triplicate measures excluding outliers were included for statistical analyses.

Statistical analyses were conducted using IBM SPSS (version 25, SPSS Inc., Chicago, IL). Assumptions for statistical models were assessed using the Shapiro–Wilk test and the Levene's test was used to check for equality of variances between groups. Data that violated the assumptions were analysed with the equivalent non-parametric test. Independent *t*-tests /Mann–Whitney U tests were used to examine between-group differences in baseline data. Mixed analysis of variance/Kruskal Wallis and Friedman's tests were used to analyse EIMD markers and dietary intake data between groups and within time points. Data sphericity was assessed with the Mauchly's test and any data that violated the Greenhouse–Geisser assumptions were corrected with Huynh–Feldt. Any significant group × time interactions were analysed using independent *t*-tests for between-group comparisons at each time point and within-group differences across time were analysed using paired *t*-tests. Bonferroni corrections were used to correct for multiple comparisons. To examine sex differences, Hedges' *g* ESs with confidence intervals were calculated using the standardised mean difference between males and females in the change from baseline to each post-exercise time-point for EIMD markers.

To assess the relationship of EIMD and body composition, total and regional body composition outcomes were independently related to the pre-peak post-exercise change in each marker of muscle damage using Spearman's *Rho* correlation coefficient. Interpretation of the correlation coefficient was as follows: 0.00–0.19 = very low association; 0.20–0.39 = low association; 0.40-0.69 = modest association; 0.70–0.89 = high association; 0.90–1.00 = very high association. Statistical significance was set at $P < 0.05$ and confidence intervals assume 95% confidence in the range of the mean. All data are reported as mean ± standard deviation (SD) unless otherwise stated.

Secondary Data

Secondary data were obtained through systematic reviewing and scoping of the relevant literature to determine the impact of dietary protein supplementation (*chapter 5*) and exercise load (*chapter 8*) on resistance EIMD. Systematic reviews are beneficial in sport science for developing practical guidelines, informing future research, and aiding practitioners, industrialists, and non-sport science academics in understanding the general consensus of a specific topic. Scoping reviews, while not as rigorous as systematic reviews, are beneficial for examining a wide body of body of literature, identifying knowledge gaps, and highlighting new directions of research (Munn et al., 2018). To avoid repetition, methodological detail and justification are provided here for *chapter 5* only, though mostly apply to *chapter 8* also.

Systematic and Scoping Review Overview

A systematic review with meta-analysis was conducted to examine the impact of dietary protein supplementation on resistance EIMD. The analysis was confined to studies published in English-language journals that met the criteria outlined in *Table A2*. The systematic literature search was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, comprising of a 27-item checklist and a 4-phase flow diagram (Moher et al., 2009) (*appendix 10*). Following these guidelines allowed for transparency in reporting of the systematic review and ensured all relevant information was included. The objectives and methods of the systematic review were pre-specified to limit bias, such as selective outcome reporting (Liberati et al., 2009). Screening and decisions on inclusion of the relevant titles, abstracts, and full text of articles were conducted by two independent reviewers (AP and LM) to reduce the possibility of rejecting relevant papers (Edwards et al., 2002). The reference lists of relevant articles were also screened, in case any eligible articles were not listed in the databases searched.

Studies were coded for variables including participant demographics; resistance exercise protocol; protein and control supplementation protocol; muscle damage assessment methods; and time points of measurements in relation to exercise, because these factors may influence EIMD or the response to protein supplementation and allow sub-group analysis. For example, a certain type of protein may only be beneficial for EIMD following a specific form of exercise, which would implicate practical recommendations. The quality/risk of bias of each study was assessed by two independent investigators (AP and LM) based on the 11-point Physiotherapy Evidence Database (PEDro) scale (*appendix 11*), which is considered a reliable and valid measure of the methodological quality of randomised controlled trials (Elkins et al., 2010; Maher et al., 2003). Low quality studies, i.e. those with high risk of bias, can exaggerate the treatment effect, thus reducing the likelihood that the overall ES produced from the meta-analysis reflects the true effect (Pildal et al., 2007).

Table A2. Eligibility criteria for studies included in the systematic review (chapter 5).

Inclusion criteria	Justification
❖ Experimental trial involving acute resistance exercise	EIMD is attenuated with repeated/chronic exercise (Balnave & Thompson, 1993) and cannot be grouped with acute exercise. Previous reviews have examined endurance and other exercise types.
❖ Measures ≥1 indirect marker of EIMD	Indirect markers (e.g. muscle function, soreness, blood biomarkers) are more commonly assessed than direct markers (e.g. MRI, ultrasound, muscle biopsy sampling) and will be assessed in the present work (*chapters 6* and *7*).
❖ EIMD is measured for ≥24 h following the initial exercise bout	Some symptoms of EIMD, including muscle soreness and increased CK activity, typically do not present until >24 h after exercise.
❖ Involves peri-exercise protein supplementation	Should not matter whether protein is consumed pre, during, or post exercise and therefore studies can be combined.
❖ Includes a non-protein dietary control group (including carbohydrate-based supplements)	A control group is required to isolate the impact of the protein supplement on EIMD and to limit bias. Carbohydrate-based supplements can be isoenergetic to the protein supplement, thus removing the influence of energy content.
❖ Involves healthy, adult participants with no known clinical condition or musculoskeletal injury	Musculoskeletal injury may limit capacity to exercise and clinical conditions and their associated medications may impact the EIMD markers. The outcomes of this review are to be applied to adults, not children.
Exclusion criteria	**Justification**
❖ Studies conducted in animals or *in vitro*	Outcomes are to be applied to humans and EIMD responses have shown to differ between animals and humans.
❖ Involve supplements containing therapeutic, pharmaceutical, or ergogenic aids (e.g. polyphenols, antioxidants, omega-3 polyunsaturated fatty acids, non-steroidal anti-inflammatory drugs)	These substances can impact EIMD and therefore confound the effect of protein supplementation.
❖ Involve physiotherapeutic methods (e.g. cryotherapy, massage, acupuncture, compression garments)	These methods can impact EIMD and therefore confound the effect of protein supplementation.

Meta-Analysis Overview

The meta-analysis was conducted by a single reviewer (AP). Each comparator group (protein vs control) within each study was treated as an independent trial, because some studies included multiple protein interventions, e.g. different protein types, doses, or timings. Separate meta-analyses were conducted at each post-exercise time-point (<24, 24, 48, 72, and 96 h) to assess whether the EIMD response to protein supplementation differed during stages of exercise recovery. Meta-analyses were conducted on 4 variables (isometric MVC, isokinetic MVC, serum/plasma creatine kinase concentration, and muscle soreness rating) because these were the most frequently measured EIMD markers among the studies included and this advanced a previous meta-analysis, which examined the impact of protein supplementation on muscle function alone following EIMD (Davies et al., 2018).

For each trial, a Hedges' *g* ES with correction for small positive bias was calculated because the within-study group sample sizes were sometimes dissimilar (Borenstein et al., 2021). ESs were calculated using mean and SD values as a percent change from baseline because EIMD markers can be highly variable among individuals, owing to factors including training status, sex, age, body mass and composition (Balnave & Thompson, 1993; Dannecker et al., 2012; Kim & So, 2018; Margaritelis et al., 2019; Roth et al., 2000a) and so using relative change data is preferred over absolute data. However, for muscle soreness, there were insufficient data to use percent change from baseline values, as baseline muscle soreness was typically not measured or reported as zero; thus, ESs were calculated using the absolute mean ± SD at each time point. Weighted ESs were calculated using the standard error of the effect and adjusted with Tau squared (τ^2).

Trial heterogeneity was assessed using Cochran's Q (Q) and I^2 (Higgins et al., 2003), as the level of heterogeneity may explain differences in outcomes between trials. Due to the moderate-substantial heterogeneity detected, a random-effect model was used to calculate the pooled ESs (Borenstein et al., 2021). The statistical significance of the pooled ES was determined by zero overlap of the 95% confidence interval range as per Cohen (1992). To identify potentially influential trials that may skew the overall treatment effect, a sensitivity analysis was conducted by performing meta-analyses with removal of each trial one at a time, which has been done previously (Schoenfeld et al., 2017_a). Trials were considered influential if their removal resulted in the pooled ES changing from significant to non-significant, or *vice versa*. Pooled ESs are reported with (displayed in forest plots) and without (in the manuscript)

inclusion of influential trials, so the reader can see the degree to which ES were altered by influential trials.

Due to high variability in the responsiveness to EIMD, the magnitude of EIMD was determined for each trial included in the meta-analyses based on the relative peak reduction from baseline in MVC as per Paulsen et al. (2012): mild = <20%, moderate = 20–50%, and severe = >50% reduction. These data were used to identify any trends in the response to protein supplementation based on the magnitude of EIMD.

Participant Information Sheet

You are invited to take part in a research project. Before you decide if you would like to take part, please read this information sheet carefully. You can also ask the lead researcher, Alice Pearson, if you have any questions (please see contact details at the end of this sheet).

Title of Project: The impact of milk protein ingestion during recovery from muscle damaging resistance exercise

What is the purpose of the research?

The purpose of the study is to investigate the impact of ingesting milk protein on muscle damage following a single bout of high-intensity resistance exercise

Why have I been invited to take part?

You have been invited to take part in this study because you are a female between the ages of 18 and 35.

You may be able to take part in the study if you:

- Are a healthy weight (BMI >18.5 and <25 kg/m^2)
- Have no previous experience in resistance training
- Are able to perform leg-based resistance exercise
- Are free from musculoskeletal injury (past 6 months)
- Are not taking any performance-enhancing or protein-based supplements
- Are free from habitual use of non-steroidal anti-inflammatory drugs (NSAIDS) i.e. twice per week for previous 1-month period
- Do not frequently engage in massage or cryotherapy
- Do not have a chronic disease or acute illness
- Eumenorrheic female (regular menstrual cycle over the past 12 months)
- Are not pregnant or breast-feeding

Do I have to take part?

Participation is voluntary – it is up to you to decide whether or not to take part. You should read this information sheet and if you have any questions you should ask the lead researcher. You should not agree to take part in this research until you have had all your questions answered satisfactorily. If you agree to take part, you will be given this participant information sheet and asked to sign a consent

form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights.

If you decide to participate in this research, you will not be able to do the following for the duration of the study:

- *Use non-steroidal anti-inflammatory drugs (e.g. ibuprofen)*
- *Perform strenuous exercise (daily activity such as walking is permitted)*
- *Engage in massage or cryotherapy*
- *Consume protein supplements, vitamin or mineral supplements, or ergogenic aids (e.g. creatine, pre-workout stimulants, steroidal drugs)*
- *Consume alcohol (for the 48 hours prior to each visit)*

What will be involved if I decide to take part in the research?

All research for this study will take part in the Human Performance Laboratory in the Durham University Sports and Wellbeing Park at Maiden Castle. You will be required to make 7 visits to the laboratory, each of which will last 40–115 minutes. The majority of testing (5 visits) will be conducted over a 1-week period with baseline assessments completed 3-weeks prior. You will be asked to provide information about your menstrual cycle prior to baseline testing. A schematic overview of the study can be found at the end of this section (*Figure 1)*.

During the first visit, you will be familiarised with the exercise equipment and demonstrated the correct lifting form. You will then complete a graded maximal strength test (known as "one rep max" or "1RM") on a leg press machine followed by a leg extension machine. During this test, the weight lifted will gradually be increased until the maximum weight you can lift for one repetition is estimated. In the second visit, your body composition will be measured using a dual energy X-ray absorptiometry scanner in the Truscott Imaging Suite. You will be required to have fasted overnight and to wear a pair of shorts and t-shirt during the scan. You will then repeat the 1RM test on the leg machines to confirm your maximal strength. Before the next visit, which will occur 3-weeks later, you will be asked to record your habitual diet and physical activity for 3 days.

On the morning of each of the remaining visits, you will be asked to consume a standardised breakfast (toast or cereal with a protein or placebo product) before attending the laboratory. During the third visit, a venous blood sample will be collected from your arm, which will later be analysed for markers of muscle damage and inflammation. You will then have the circumference of your upper leg measured and the range of motion of your leg measured to assess flexibility. You will be asked to rate your muscle soreness; firstly, by the Pressure-Pain Threshold test, during which a small rod-like device

is pushed against your quadricep muscle with increasing pressure until pain is indicated and secondly, by a visual analogue scale. You will then perform a single bout of resistance exercise on the leg press and leg extension machines, with each set performed until failure (i.e. when you can no longer lift the weight). After the exercise, another blood sample will be taken and the assessments of limb circumference, range of motion, and muscle soreness will be repeated. You will then consume another protein or placebo snack before leaving the laboratory and be given a further two snacks to consume at home. The dietary condition you are assigned to (protein or placebo) will be done at random.

During the fourth, fifth, sixth (consecutive days after visit 3), and seventh visit (one week after visit 3) repeated blood samples will be collected, and we will again measure your limb circumference, leg range of motion, and muscle soreness. You will not have to perform any exercise during visits four and five. During visits six and seven, you will be asked to repeat the maximal strength test (1RM). Again, you will be asked to consume the protein or placebo snack with breakfast, after testing, and twice more in the day over the study period (4 snacks per day for 4 days).

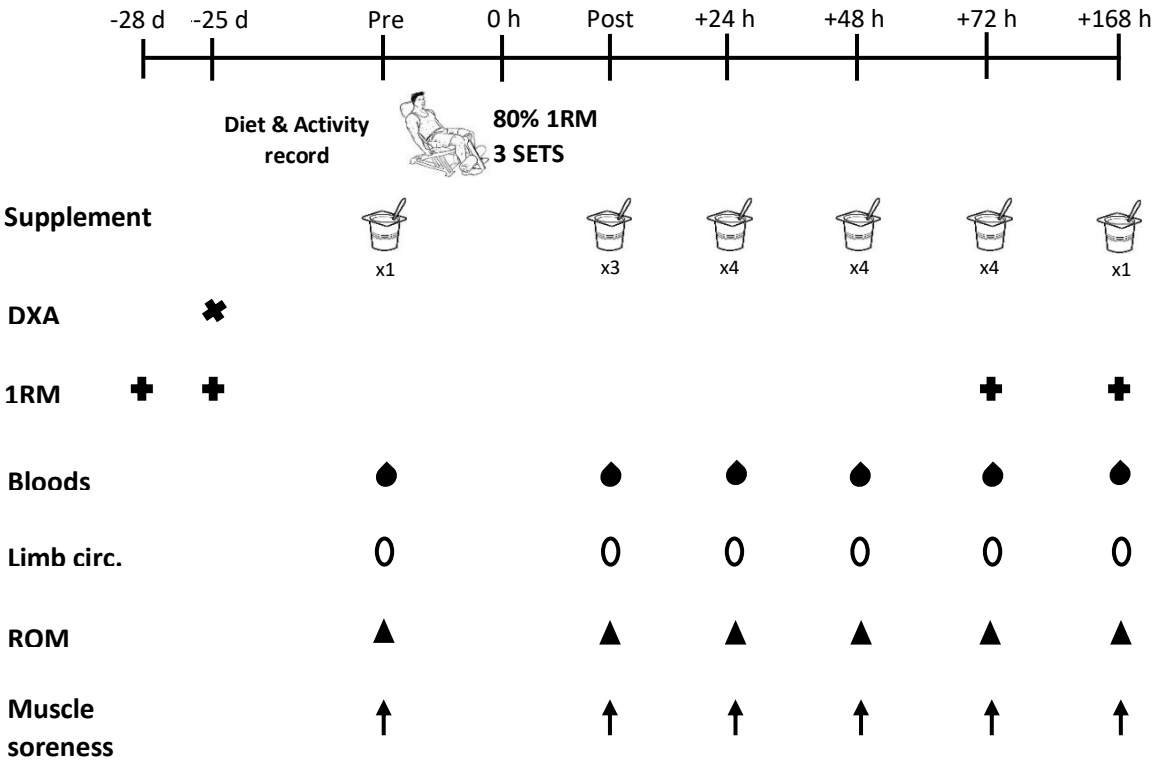


Figure 1. Schematic overview of study design.

Note: DXA = dual-energy X-ray absorptiometry; 1RM one repetition maximum; limb circ. = limb circumference; ROM = range of movement. Protein supplement = milk-based product (yoghurt) providing 20 g of protein; Placebo = isoenergetic carbohydrate-based product providing <1 g of protein.

What are the benefits and risks of taking part?

There are a few risks that you need to be aware of in your consideration of participating in this research. These are:

- The maximum strength test is performed to muscular failure, which may cause dizziness, nausea, and muscle soreness. You will be shown the correct lifting technique and perform an appropriate warm-up to reduce risk of injury and will be supervised at all times.
- The incision of the needle to draw blood can cause discomfort and may cause minor bruising and tenderness. There is a small risk of infection through use of the needle, but this risk will be minimised by the blood samples being collected by a trained phlebotomist using the appropriate personal protective equipment.
- There is a small dose of radiation associated with DXA. The total amount of radiation for participation in the complete study is <2 µSv. This is less than half a day of natural background radiation (~3.6 µSv). The dose associated with participation in this study has been formally assessed by a Medical Physics Expert and a Clinical Radiation Expert. We will not perform a DXA scan on you if you are a female who is pregnant or if you suspect that you may be pregnant.

There are several benefits to taking part in this research, which include:

- You will receive a body composition analysis, which will tell you your percentage body fat, amount of muscle mass, and bone mass.
- You will receive a dietary analysis conducted by a registered sport nutritionist – this will tell you how many calories you consume; how much fat, protein, and carbohydrates your diet contains, and if you are meeting your micronutrient requirements.
- You will be informed of your maximal leg strength, which may be useful for personal progress

What steps are being taken for preventing the spread of Covid-19?

Prior to each laboratory visit, you will be asked to conduct a Covid-19 lateral flow test. This procedure is completed by yourself and the results are available within 30 minutes. If you receive a positive result, you will be instructed to leave the university premises and self-isolate in accordance with the most recent government guidance. If, within 14 days prior to your appointment, you have experienced any symptoms of Covid-19 or have been in close contact with anyone who has tested positive for Covid-19, you must not attend. Instead, you should rearrange for a later date. We are maintaining a list of all visitors to the research site in order to support the 'track and trace' system.
During your appointment, a 2-metre distance will be observed between the researcher and yourself apart from when the physical tests are carried out. The laboratory is installed with a ventilation system

that is monitored to ensure air quality is maintained. At all times, hand hygiene will be followed, and hand sanitiser stations are in place throughout the building and equipment will be sanitised before and after use. A face covering will be worn by the researcher and, unless except, a face covering should be worn by yourself when in the University building.

How will confidentiality be assured?

- All data files will be stored on computers or laptops with passwords that are only known by the research team and any hard copies will be stored in a locked filling cabinet which only the researchers can access.
- Your name will not be used to label data files and samples collected. Instead, you will be given a participant number, which will only be known by you and the research team. This way none of the other participants or anyone outside of the research team will be able to identify which test data belongs to you.
- The data presented in the written manuscript will not be identifiable to you

Please refer to the 'Privacy Notice' provided in your participant information pack for more information.

What will happen to the results of the research?

The results from the research will be included in a PhD thesis, published in an academic journal, and presented at academic conferences.

If you have any questions related to the project, please contact the lead researcher:

Alice Pearson

Email address: alice.g.pearson@durham.ac.uk

Supervisor: Dr Karen Hind

Address: 42 Old Elvet, DH1 3HN

Email address: karen.hind@durham.ac.uk

If you would like to take part and are happy with the answers to your questions, please complete and sign the enclosed Informed Consent Form.

Privacy Notice

This notice provides you with the privacy information that you need to know before you provide personal data for the particular purpose(s) stated below. Additional information about the University's responsibilities for data protection and your rights in relation to personal data can be found in the University's generic privacy notice, available at https://www.dur.ac.uk/research.innovation/governance/privacynotice/generic/.

Title of Project: The impact of milk protein ingestion during recovery from muscle damaging resistance exercise

Type(s) of personal data collected and held by the Researcher and method of collection:

Personal data will be collected through self-reported questionnaires. These data will include your name, age, sex, ethnicity, physical activity level, and general health i.e. current illness.

Lawful Basis:

- The University's core purpose includes undertaking research in the public interest. Processing of your data is carried out as part of this core purpose.
- Your data will be processed in accordance with the consent you give for the use of your data, should you agree to participate in the project.

How personal data is stored:

- All data will be kept strictly confidential to the research team.
- All data files will be stored on computers or laptops with passwords that are only known by the research team and any hard copies will be stored in a locked filling cabinet which only the researchers can access.
- Your name will not be used to label data files and samples collected. Instead, you will be given an anonymous participant number, which will only be known by you and the research team. This way none of the other participants or anyone outside of the research team will be able to identify which test data belongs to you.
- The data presented in the written manuscript will not be identifiable to you

How personal data is processed:

Personal data is used for descriptive purposes and to analyse responses according to certain criteria. Information will be entered into a database for analysis. After six months the data will be completely anonymised and the original records, including any information which can identify you personally, will be destroyed.

Withdrawal of data:

You can request withdrawal of your data up until it has been fully anonymised. Once your data is anonymised, it will not be possible to identify you from any of the data we hold.

Who the Researcher shares personal data with:

Personal data will only be shared among the research team. Anonymised personal data will be included in any publications of the research (with your permission, as stated on the consent form), but these data will not be identifiable to you.

How long personal data is held for:

We will hold personal data for six months, after which it will be anonymised.

How to object to the processing of your personal data:

If you have any concerns regarding the processing of your personal data, or you wish to withdraw your data from the project, contact the lead researcher, Alice Pearson, using the contact details below.

If you require further information, please contact:

Lead researcher: Alice Pearson

Email: alice.g.pearson@durham.ac.uk

Supervisor: Dr Karen Hind

Address: 42 Old Elvet, DH1 3HN

Email: karen.hind@durham.ac.uk

Appendix 4. *Pre-participation screening questionnaire*

Pre-Participation Screening Questionnaire

Just a few questions to check whether you're eligible to take part in the study titled 'Sex differences in resistance exercise-induced muscle damage: The impact of milk protein ingestion'

* Required

* This form will record your name, please fill your name.

1. Can you confirm you are aged 18-35 years? *

- ◯ Yes
- ◯ No

2. Have you performed resistance exercise (weight lifting) in the previous 6 months? *

- ◯ Yes
- ◯ No

3. Do you habitually (3 times per week) perform aerobic exercise with a large eccentric component, e.g., downhill running, box jumps? (If you perform running on mostly flat surfaces, answer 'no') *

- ◯ Yes
- ◯ No

4. Do you have any current or recent (previous 6 months) musculoskeletal injury? *

- ◯ Yes
- ◯ No

5. Are you able to perform leg-based resistance exercise? *

- ◯ Yes
- ◯ No

6. Are you able to consume dairy products? (i.e., not lactose-intolerant or vegan) *

- ◯ Yes
- ◯ No

7. Do you suffer from any chronic illness? *

- ◯ Yes
- ◯ No

8. To the best of your knowledge do you have any infectious diseases? *

- ◯ Yes
- ◯ No

9. In the past 2 weeks, have you had any symptoms of Covid-19 or been in contact with anyone who has since tested positive for Covid-19? *

- ◯ Yes
- ◯ No

10. If you have not been fully vaccinated against Covid-19, would you be willing to perform a Lateral Flow Test (LFT) prior to commencing the study? *

- ◯ Yes
- ◯ No

11. (Females only) Have you had a regular menstrual period for the past 12 months?

- ◯ Yes
- ◯ No

12. (Females only) Are you currently or suspect you may be pregnant or breastfeeding?

- ◯ Yes
- ◯ No

13. Do you regularly consume (2 times per week) performance-enhancing (e.g., creatine monohydrate, steroidal drugs), antioxidant (e.g., vitamin C, Montmorency cherry juice), or anti-inflammatory (e.g., ibuprofen) substances? *

- ◯ Yes
- ◯ No

14. Do you regularly (2 times per week) engage in cryotherapy, massage, or foam-rolling? *

- ◯ Yes
- ◯ No

15. Should you decide to participate in this study, would you be willing to abstain from the following during the study period:
    - Use non-steroidal anti-inflammatory drugs (e.g., ibuprofen) for the 48 hours prior to each laboratory visit
    - Strenuous exercise (daily activity such as walking is permitted)
    - Massage, cryotherapy, or foam-rolling
    - Consumption of protein supplements, vitamin or mineral supplements (those with anti-oxidant or anti-inflammatory properties), or ergogenic aids (e.g., creatine, pre-workout stimulants, steroidal drugs)
    - Consumption of alcohol (for the 48 hours prior to each visit) *

- ◯ Yes
- ◯ No

Consent Form

Project title: The impact of milk protein ingestion during recovery from muscle damaging resistance exercise

Researcher: Alice Pearson
Department: Sport and Exercise Sciences
Contact details: alice.g.pearson@durham.ac.uk
Supervisor name: Dr Karen Hind
Supervisor contact details: karen.hind@durham.ac.uk

This form is to confirm that you understand what the purposes of the project, what is involved and that you are happy to take part. Please initial each box to indicate your agreement:

I confirm that I have read and understand the Information Sheet dated [__/__/__] and the Privacy Notice for the above project.	
I have had sufficient time to consider the information and ask any questions I might have, and I am satisfied with the answers I have been given.	
I understand who will have access to personal data provided, how the data will be stored and what will happen to the data at the end of the project.	
I agree to take part in the above project.	
I understand that my participation is entirely voluntary and that I am free to withdraw at any time without giving a reason.	
I understand that anonymised (i.e. not identifiable) versions of my data may be archived and shared with others for legitimate research purposes.	
I am not currently participating in another research project	
I have not participated in a research project involving dietary and/or exercise intervention in the previous 6 months	
To my knowledge, I am not currently pregnant nor breastfeeding	

Participant's Signature_____ Date_____

(NAME IN BLOCK LETTERS)_____

Researcher's Signature_____ Date_____

(NAME IN BLOCK LETTERS)_____

Appendix 6. *Readiness to exercise questionnaire*

Health & Readiness to Exercise Questionnaire

As best as you can, please answer these questions about your general health to ensure you are able to perform the resistance exercise involved in the study titled 'Sex differences in resistance exercise-induced muscle damage: The impact of milk protein ingestion'. All answers will be kept confidential and only accessible by the lead researcher. If you are unsure about anything, please contact me: alice.g.pearson@durham.ac.uk

* Required

* This form will record your name, please fill your name.

1. Please provide your full name *

2. What is your biological sex? *

- ◯ Male
- ◯ Female

3. What is your date of birth? *

4. Please provide your email address *

5. Please provide a contact telephone number (the researcher may only contact you during the study period for reminders about appointments etc.)

6. Has your doctor ever said you have a heart condition and/or should only participate in medically supervised physical activity? *

- ◯ Yes
- ◯ No

7. Do you often feel pain in your chest during mild physical activity? *

- ◯ Yes
- ◯ No

8. Do you suffer with palpitations? *

- ◯ Yes
- ◯ No

9. Do you often experience dizziness or fainting? *

- ◯ Yes
- ◯ No

10. Have you ever been told that you have high blood pressure or are you taking medication for blood pressure or any other heart condition? *

- ◯ Yes
- ◯ No

11. Do you experience shortness of breath during only mild exertion? *

- ◯ Yes
- ◯ No

12. Do you suffer from either asthma or Diabetes Mellitus? *

- ◯ Yes
- ◯ No

13. Do you have any liver, kidney or thyroid disorders? *

- ◯ Yes
- ◯ No

14. Do you have epilepsy or suffer from seizures? *

- ◯ Yes
- ◯ No

15. Do you have any existing bone or joint problems that could be made worse by physical activity? *

- ◯ Yes
- ◯ No

16. Are you currently taking any prescribed medication? If so, what? *

17. Have you recently undergone surgery or suffered a musculoskeletal injury (previous 6 months)? If yes, please explain... *

18. Are you currently ill in any way? (e.g., common cold, sickness bug) If yes, please explain... *

19. Are you aware of any other reason why you should not participate in physical exercise without medical supervision? If so, please explain... *

20. Are you currently on a medically prescribed diet? If yes, please explain... *

21. Do you have any food allergies? If yes, please explain... *

22. Do you have any specific dietary restrictions (e.g., gluten-free, vegan)? If yes, please explain... *

23. Do you currently take any dietary supplements? If yes, please explain... *

24. (Females only) Are you currently pregnant or breastfeeding?

- ◯ Yes
- ◯ No
- ◯ Maybe

25. (Females only) Have you had a regular menstrual period for the past 12 months?

- ◯ Yes
- ◯ No

26. (Females only) If applicable, approximately when was the date of your last menstrual period? (The date your period started)

Appendix 7. *Three-day diet and activity record template and example*

3-Day Diet & Activity Diary

	Breakfast	Lunch	Dinner	Snacks	Drinks
Day 1					
Activities					
Day 2					

Activities					
Day 3					
Activities					

Diet & Activity Diary Example

Guidance:

- Try to fill out the diary after each meal, rather than at the end of the day so you don't forget anything.
- Remember to include drinks, added sugars, and condiments
- State if the food item is a fat-free/low-fat/low-sugar version
- If the weight of a food item is known (i.e. from the packaging) please include this
- If the weight is not known, please estimate through 'household measures' (i.e. 1 tsp, a handful, a fistful, a large bowl)
- Be honest! I'm not judging you!

	Breakfast	Lunch	Dinner	Snacks	Drinks
Day 1	1 sachet of instant porridge with 200 mL semi-skimmed milk, 1 tsp honey, 1 banana, Mug of coffee with semi-skimmed milk & 1 tsp sugar	Chicken salad sandwich on white bread with butter, Packet of Walkers crisps, Can of diet coke (330 mL)	3 pork sausages, fistful of mashed potato, 3 heaped tbsp of peas, gravy	1 apple with 1 tbsp peanut butter, 2 chocolate digestive biscuits	2 cups of tea with milk & 1 tsp sugar, Water throughout day
Activities	Cycled 10 mins to university, 4 hours sitting at desk, 20 mins walk at lunch, 3 hours sitting at desk, cycled 10 mins home, 1 hour household chores (cooking, cleaning), 2 hours sitting on sofa, 8 hours sleep.				

Important Information

Pre-DXA scan Preparation Guidance

The following pre-scan preparation protocol ensures that your DXA scan results will be as accurate as possible.

Please follow this guidance before attending your DXA scan appointment and if you have any questions, please contact: alice.g.pearson@durham.ac.uk.

- My appointment is before 11 am = Please **fast overnight** (last meal no later than 10 pm)
- My appointment is after 11 am = Please fast for 5 hours prior to your scan appointment
- Drink 500 mL of water 3 hours before your appointment (only small sips of water after this)
- No moderate-vigorous exercise in the 12 hours before your appointment
- No caffeine in the 5 hours before your appointment
- No alcohol in the 24 hours before your appointment
- Please wear/bring light weight, close-fitting clothing that does not contain metal, underwire, plastic or reflective strips. Ideal clothing would be **lightweight shorts and t-shirt**.

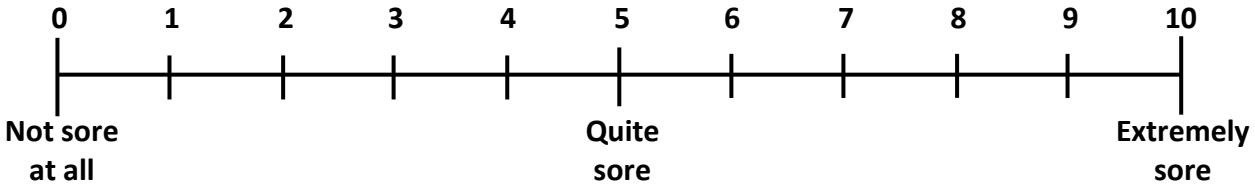
You may bring a snack with you for after your scan.

Appendix 9. *Visual rating scale*

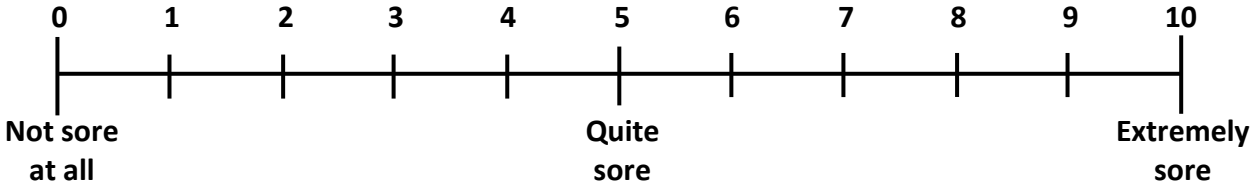
Self-perceived Muscle Soreness

After having performed a standard bodyweight squat, how would you rate the muscle soreness in your:

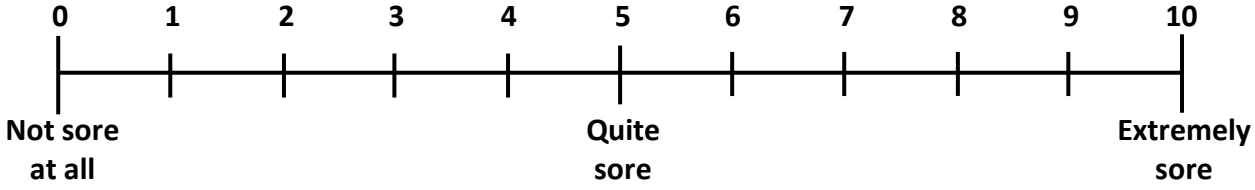
1) Quadriceps



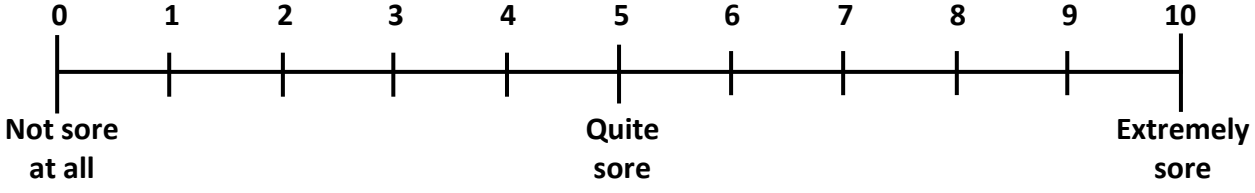
2) Hamstrings



3) Glutes



4) Calves



Appendix 10. *PRISMA checklist*

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention	

Section and Topic	Item #	Checklist item	Location where item is reported
methods		characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	

Section and Topic	Item #	Checklist item	Location where item is reported
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

Appendix 11. *PEDro scale*

PEDro scale

1.	eligibility criteria were specified	no ❑ yes ❑ where:
2.	subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received)	no ❑ yes ❑ where:
3.	allocation was concealed	no ❑ yes ❑ where:
4.	the groups were similar at baseline regarding the most important prognostic indicators	no ❑ yes ❑ where:
5.	there was blinding of all subjects	no ❑ yes ❑ where:
6.	there was blinding of all therapists who administered the therapy	no ❑ yes ❑ where:
7.	there was blinding of all assessors who measured at least one key outcome	no ❑ yes ❑ where:
8.	measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	no ❑ yes ❑ where:
9.	all subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by "intention to treat"	no ❑ yes ❑ where:
10.	the results of between-group statistical comparisons are reported for at least one key outcome	no ❑ yes ❑ where:
11.	the study provides both point measures and measures of variability for at least one key outcome	no ❑ yes ❑ where: