A Comparison of Systemic Inflammation Pre- and Post-Exercise Between Vegans and Omnivores

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Abstract

Introduction: Exercise-induced muscle damage (EIMD) triggers an inflammatory response essential for muscle repair and recovery. The impact of habitual dietary patterns on this response is not well understood.

Aim: This study investigated whether nutrient source (i.e., vegan or omnivorous diet) has an impact on the systemic inflammatory response or muscle damage markers following an eccentric exercise protocol.

Methods: Three vegans (2 males; 32.3 ± 5.78 years; 63.4 ± 16.9 kg; 173.7 ± 9.5 cm; 20.8 ± 3.4 BMI) and seven omnivores (6 females; 25.0 ± 5.34 years; 70.9 ± 8.5 kg; 167.9 ± 8.2 cm, 24.1 ± 2.5 BMI) underwent an eccentric exercise protocol involving 200 drop jumps. Muscle damage marker CKM, and inflammatory biomarkers IL-1β, IL-6 and IL-10 were analysed from plasma collected at baseline and 0 h, 1 h, 3 h, 24 h, 48 h, 72 h post-exercise. Participants followed a macronutrient-matched meal plan and consumed a standardised meal post-exercise.

Results: IL-6 increased over time (P= 0.005), however there was no difference between diets (P= 0.225). IL-1β, IL-10 and CKM did not change over time (P= 0.371, P=0.097 and P=0.491, respectively) or differ between diets (P= 0.925, P= 0.821 and P= 0.296, respectively). Dietary analysis of participants' habitual diets showed vegans had significantly lower intakes of cholesterol (P= 0.007), and dietary folate equivalents (P= 0.040). Dietary analysis of participants' intake during the trial showed vegans had significantly lower intakes of cholesterol (P= 0.010), niacin equivalent (P= 0.028), and vitamin B6 (P= 0.032). Daily intake of fibre, folate, vitamin A, omega-3 polyunsaturated fatty acids, selenium, calcium, and zinc were below recommended levels for the vegans. Whereas daily intake of calcium was below recommended levels for the omnivores.

Conclusion: The study found no significant differences in systemic inflammatory or muscle damage markers between habitual vegan or omnivorous diets following eccentric exercise. Despite significant differences in nutrient intake between diets, the eccentric exercise protocol was not strenuous enough to produce notable systemic stress detectable by the biomarkers measured. The small sample size limited the study's power, highlighting the need for further research with larger samples and more comprehensive assessments, including, for example, muscle ultrasound for assessment of muscle damage, additional inflammatory biomarkers (e.g., IL-4 and IL-1ra), and plasma lipid panels and plasma nutrient levels. Future studies should continue to explore the effects of whole dietary patterns on muscle recovery and inflammation without inducing nutrient deficiencies or excessive supplementation.

Keywords: Exercise-induced muscle damage (EIMD), inflammatory response, habitual diet, vegan, omnivore, eccentric exercise, IL-6, CKM, IL-10, IL-1β.

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Abbreviations

Abbreviation or Symbol	Definition
AI	Adequate intake
BCAA	Branched-chain amino acids
BMI	Body mass index
CHO	Carbohydrate
CMJ	Counter movement jump
CK	Creatine kinase
CKM	Creatine kinase (muscle)
COX enzymes	Cyclooxygenase
DOMS	Delayed onset muscle soreness
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
EAAs	Essential amino acids
E-C coupling	Excitation-contraction coupling
EER	Estimated energy requirements
EIMD	Exercise-induced muscle damage
EPA	Eicosapentaenoic Acid
HDL	High-density lipoprotein
hsCRP	High-sensitivity C-reactive protein
IFN-γ	Interferon-γ
IGF-1	Insulin-like growth factor-1
IL-1	Interleukin-1
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-1β	Interleukin-1β
IL-1ra	Interleukin-1 receptor antagonist
iNOS	Inducible nitric oxide synthase
LDL	Low-density lipoprotein
MRI	Magnetic resonance imaging
MVIC	Maximum voluntary isometric contraction
n-3 PUFA	Omega-3 polyunsaturated fatty acid
NAD+	Nicotinamide adenine dinucleotide
NADPH	
NFAT	Nuclear factor of activated T cells
NSAIDs	Nonsteroidal anti-inflammatory drugs
RDI	Recommended daily intake
ROS	Reactive oxygen species
TGF-β	Transforming growth factor-β1
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Chapter 1 Introduction

1.1 Background

The acute inflammatory process following exercise-induced muscle damage is essential for muscle tissue repair, and therefore, recovery of performance. This period of inflammation is required to bring excess fluid and immune cells to the site of injury. During the pro-inflammatory phase, the immune cells phagocytose damaged muscle fibres, and muscle stem cells activate and proliferate. Once the anti-inflammatory phase is triggered, the muscle stem cells differentiate to repair damaged fibres, allowing for recovery of strength and function.

Exercise-induced muscle damage (EIMD) causes myofibre cell content leakage and the resulting cell debris trigger the immune system and subsequent inflammatory process. Lengthening eccentric exercises causes muscle filament overextension, the overlap between actin and myosin is reduced and the sarcomeres become overstretched (Fatouros & Jamurtas, 2016; Owens et al., 2019). At muscle relaxation, the sarcomeres may not return to their original state, causing damage to the surrounding membranes, allowing an influx of Ca^{2+} into the cell and muscle proteins to leak into circulation (Fatouros & Jamurtas, 2016). The resident immune cells (neutrophils and mast cells) activate and degranulation of their pro-inflammatory cytokines triggers the acute inflammatory response (Yang & Hu, 2018). These cytokines activate and attract peripheral immune cells (more mast cells and neutrophils, as well as macrophages) to the site of injury. Neutrophils and macrophages phagocytose damaged muscle cells and cellular debris, they also release reactive oxygen species (ROS) which further degrade muscle tissue (Owens et al., 2019). Muscle degeneration is followed by the regenerative phase of muscle recovery.

Muscle tissue regeneration is facilitated by stem cells, known as satellite cells, which are the precursor to skeletal muscle cells. These cells remain quiescent until activated by EIMD (Owens et al., 2019; Yang & Hu, 2018). Upon activation, they re-enter the cell cycle and begin to proliferate (Owens et al., 2019; Yang & Hu, 2018). This activation occurs during the pro-inflammatory phase when M1 macrophages release pro-inflammatory cytokines TNF-α, IL-6 and IL-1β; these cytokines attract satellite cells to the damaged tissue and promote their proliferation (Chazaud, 2016; Pillon et al., 2013). As the repair process continues, M1 macrophages switch to the M2 anti-inflammatory form, releasing cytokines IL-4, IL-10 and IL-13 (Peake et al., 2016; Tidball & Villalta, 2010). These cytokines help repress the local pro-inflammatory response, marking the beginning of the anti-inflammatory phase (Yang & Hu, 2018). During this phase, satellite cells either fuse to form new myofibres or merge with existing myofibres to repair damage. These newly formed myofibres mature, increasing in size until they are indistinguishable from undamaged myofibres (Bentzinger et al., 2013; Yin et al., 2013). It is at this final stage of muscle regeneration that contractile force is fully restored, allowing for complete recovery of performance.

The management of inflammation following exercise is complex, as both excessive pro-inflammatory responses and over-suppression can negatively impact muscle recovery and adaptation. Prolonged inflammation is marked by excessive reactive oxygen species production and may contribute to fibrosis, causing maladaptive muscle remodelling (Chazaud, 2016; Peake et al., 2016). Therefore, the duration of the pro-inflammatory phase must be carefully balanced to avoid maladaptive recovery. While modulating the pro-inflammatory response might seem beneficial, excessive suppression can

impair muscle regeneration as evidenced by inconsistent results with NSAIDs (nonsteroidal anti-inflammatory drugs), which may hinder satellite cell activity and muscle recovery (Howatson & van Someren, 2008; Urso, 2013). Although some studies show NSAIDs reducing muscle damage markers, others report increased damage, likely due to dosage differences (Donnelly et al., 1990; Rahnama et al., 2005). Chronic NSAID use in older adults may improve exercise adaptation by suppressing low-grade systemic inflammation (Trappe et al., 2010). Alternative strategies like cold water immersion and adequate sleep show promise in reducing inflammation and aiding recovery but may also interfere with beneficial exercise adaptations (Irwin et al., 2006; Nédélec et al., 2013). The role of nutrition in managing inflammation and aiding muscle recovery is also crucial and warrants detailed exploration.

Various nutrients, including proteins, carbohydrates, fats and antioxidants, play indispensable roles in muscle repair and inflammation modulation. Skeletal muscle mass and regeneration are regulated by muscle protein synthesis and breakdown, which is influenced significantly by dietary protein intake (Kerksick et al., 2021; Pinckaers et al., 2022). Dietary protein provides the essential amino acids (EAAs) crucial for muscle protein synthesis, therefore, EAAs are vital for muscle regeneration, growth and overall function (Arentson-Lantz & Kilroe, 2021; Monteyne et al., 2023). Animal-based proteins provide a complete array of EAAs and are typically well-digested; whereas, plant-based sources often have lower digestibility and may lack some EAAs like lysine and methionine (Arentson-Lantz & Kilroe, 2021; Kreider & Campbell, 2009; Pinckaers et al., 2021). Plant-based proteins may also be more readily converted to urea, reducing the amino acids available for muscle protein synthesis, thus limiting their effectiveness in promoting muscle recovery post-exercise (van Vliet et al., 2015). Carbohydrates are the primary energy source during high-intensity exercise, delaying fatigue and improving performance by maintaining muscle and liver glycogen stores (Ivy, 2004). Post-exercise carbohydrate intake is needed to replenish glycogen stores, promote recovery and reduce muscle damage (Roy et al., 1997). Dietary fats exhibit a dual relationship, saturated fats can exacerbate inflammation, whereas unsaturated fats demonstrate anti-inflammatory properties (Franco-de-Moraes et al., 2017). Antioxidants encompass vitamins and minerals and serve as crucial defences against exercise-induced muscle damage (Bell et al., 2015). Zinc, a mineral found in foods like meat and legumes, contributes to muscle repair and mitigates inflammation (Gammoh & Rink, 2017).

The inflammatory response following exercise has been well studied in omnivores, with some research extending to vegetarians but there is limited data on vegans. A vegan diet is solely plant-based; high in vegetables, fruit, grains, and legumes, excluding all animal products (Eichelmann et al., 2016). Whereas, a vegetarian diet includes the animal products eggs and dairy (Eichelmann et al., 2016). This is significant as there has been an indication that a high intake of saturated fat (found in dairy and eggs) can induce inflammation (Franco-de-Moraes et al., 2017). Evidence suggests that vegetarians have lower levels of systemic inflammation compared to omnivores (Franco-de-Moraes et al., 2017). Therefore, it is reasonable to conclude that vegans may also exhibit lower levels of inflammatory markers than omnivores, and potentially even lower levels than vegetarians, due the differences in dairy and egg consumption. However, more research is required as it is not well understood whether these lower systemic levels optimise the muscle inflammation response post-exercise and enhance recovery.

It is important to note that many studies focus on the supplementation of specific nutrients rather than whole foods or entire dietary patterns, which can affect nutrient bioavailability and absorption. In many studies, a group given dietary supplements is compared to a control group assigned a nutrient-deficient diet (Bell et al., 2015; Clifford et al., 2016; DiLorenzo et al., 2014; Tartibian et al., 2011). However, comparing nutrient-deficient and highly supplemented groups can lead to misleading conclusions because deficiency and excess nutrients don't have opposite effects. For instance, while iron deficiency causes anaemia, excess dietary iron does not increase red blood cell count (British Nutrition Foundation, 1995). As a result, neither group serves as an appropriate reference for typical conditions. Using a control group with a 'habitual' or average diet would provide a more realistic basis for assessing the effects of supplements. Furthermore, variability in exercise type (endurance versus resistance training), timing of supplementation (before or after exercise), and duration of intervention (acute versus chronic) add complexity when interpreting study results. These issues highlight the importance of considering ecological validity (how well study designs mimic real-life conditions) when evaluating the impact of dietary interventions on muscle recovery and inflammation.

The complexity of exercise-induced inflammation and muscle recovery emphasises the importance of understanding how dietary patterns influence these processes. While extensive research has explored the inflammatory response in omnivores and to a lesser extent, vegetarians, data on vegans remain sparse. Additionally, the impact of habitual diets compared to supplemental interventions on inflammation has not been thoroughly examined. This study aims to bridge these gaps by comparing the effects of vegan and omnivorous diets on inflammation and muscle damage post-exercise, with a focus on habitual dietary intake. By addressing these knowledge gaps, the study will provide valuable insights into how dietary sources influence inflammation and contribute to more informed nutritional recommendations for optimising recovery post-exercise.

1.2 Purpose of the Study

The purpose of this study is to investigate the differences between vegans and omnivores in the time course of inflammatory cytokines and markers of muscle fibre damage after unaccustomed eccentric exercise bouts. This study endeavours to match macronutrient intake and the EIMD across participants with a strict exercise protocol and monitor the inflammatory response, this will add further insight into the relationship between veganism and post-exercise inflammation.

1.3 Aim and Objectives

The aim of this study is to determine if the nutrient source (i.e., vegan or omnivorous diet) has an impact on the systemic inflammatory response or muscle damage markers post-exercise among healthy adults based in Auckland, New Zealand. This study has three main objectives:

Objective one: compare the inflammatory response between participants by measuring plasma cytokine (IL-6, L-10, and IL-1β) levels at hours 0, 1, 3, 24, 48, and 72 post-exercise protocol.

Objective two: analyse participants' habitual diets and match macronutrient intake between the vegan and omnivore groups based on participants' usual intake for 72 hours during the trial. Energy at individual requirements, 1.4 - 1.6g/kg protein, 55% of energy in CHO, remainder energy in fat.

Objective three: compare the muscle damage between participants following exercise by measuring plasma CKM levels at hours 0, 1, 3, 24, 48, and 72 post-exercise protocol.

1.4 Hypotheses

It is hypothesised that the vegan dietary group will have lower muscle damage biomarkers following eccentric exercise, and lower inflammatory biomarkers both prior and following eccentric exercise.

1.5 Structure of Thesis

This thesis is comprised of four chapters. This first chapter briefly introduces the topic and provides the justification for this study. Chapter two is a critical review of current literature relating to exercise-induced muscle damage, the role of inflammation in muscle recovery, the effects of nutrients on inflammation, and the differences in inflammation between vegans and omnivores. Chapter three is a complete presentation of this study in manuscript format. The fourth and final chapter provides a summary of this study, including any limitations, and recommendations for future research.

1.6 Researcher Contributions

Table 1.1: Researchers' contributions to this study

Sarah Duncan Dietetic Student	Author of this thesis. Researcher. Obtained ethics. Designed study protocol and intervention. Recruited participants. Conducted trials, including the muscle damaging protocol, force and performance tests, and pain tolerance tests. Cleaned and handled data. Completed statistical analysis. Interpreted and discussed results.
Ben Duncan Dietetic Student	Co-researcher. Obtained ethics. Designed study protocol and intervention. Recruited participants. Conducted trials, including the muscle damaging protocol, force and performance tests, and pain tolerance tests. Cleaned and handled nutritional data. Completed statistical analysis.
Kaio Vitzel Main supervisor	Academic supervisor. Provided academic support and feedback for thesis. Provided guidance on ethics application, study protocol including intervention, blood analysis and statistical analysis.
Andy Foskett Co-supervisor	Academic co-supervisor. Provided guidance on ethics application, study protocol and intervention.
Giovanna Santana PhD Student	Blood analysis support: IL-6, IL-10 & IL-1β.
Henry Jones PhD Student	Blood analysis support: CKM.

Chapter 2 Literature Review

Exercise can cause muscle fibre damage, which is important for adaptation and strength gains. Before recovery of function can be achieved, injured muscle tissue may need to undergo degeneration, inflammation, and regeneration (Urso, 2013). Within the degeneration stage, damage occurs across a primary and secondary phase. Primary is the damaging stimulus, and secondary occurs from calcium influx and leukocyte activity. During the inflammatory stage, a pro- and anti-inflammatory response is observed, and fibrosis can arise if too long is spent within the pro-inflammatory phase, typically in cases of severe damage. The regenerative stage is multi-faceted and occurs across the inflammatory response.

This chapter will explore these stages in detail, as well as the inflammatory differences between vegans and omnivores, and review existing literature on the nutritional strategies to enhance this recovery process.

2.1 Exercise-Induced Muscle Damage

Exercise and the subsequent muscle damage are important for adaptations in strength and performance for athletes and non-athletes alike. This subsection will define EIMD, explore the causes and the symptoms including their implications, discuss the mechanisms, and outline the best measures of EIMD.

2.1.1 What is exercise-induced muscle damage, and what causes it?

EIMD is the disruption of muscle fibres and subcellular structures resulting from unfamiliar and/or high-intensity exercise (Clarkson & Hubal, 2002; Fatouros & Jamurtas, 2016). The mechanical strain on the muscle fibres causes damage of varying severity depending on the type, intensity, and duration of exercise, as well as the individual's trained state as unaccustomed exercise causes more damage (Markus et al., 2021; Owens et al., 2019). The primary causes of EIMD include eccentric muscle contractions, high-intensity plyometric exercises, and endurance exercises (Hyldahl & Hubal, 2014). Moderate to severe muscle damage is more prevalent in eccentric exercises, where the muscle is lengthened under force, and the fibres are overstretched (Paulsen et al., 2012).

2.1.2 Exercise-induced muscle damage mechanisms

Exercise-induced muscle damage occurs in two stages: primary and secondary damage. Primary damage is caused by a damaging activity, such as eccentric exercise, and secondary damage occurs from calcium influx and leukocyte activity during the pro-inflammatory immune response.

During primary damage, mechanical loading from eccentric exercises lengthens the muscle under force, damaging the tissue by myofibril overextension. Muscle fibres (or muscle cells) are comprised of bundles of myofibrils, within which are repeating functional units of sarcomeres (Biga et al., 2020). The sarcomere block is responsible for contracting and lengthening the muscle fibres; this is achieved by the overlapping protein filaments, actin and myosin, which slide tighter together to contract or further apart to lengthen (Biga et al., 2020). A well-supported hypothesis posits that in eccentric movements, these filaments are drawn apart and dependent on the level of force, drawn beyond myofilament overlap. (Owens et al., 2019). This theory is termed "popped sarcomeres" and results in Z-line streaming, the passive Z-line structure where the actin filaments are anchored is deformed

(Proske & Morgan, 2001; Sorichter et al., 1999). It is believed that as more sarcomere units are overstretched, this can lead to myofibre and sarcolemma (muscle cell membrane) damage, the opening of the stretch-activated membrane channels (Hyldahl & Hubal, 2014), and failure of the excitation-contraction (E-C) coupling process. This physical damage and disruption are attributed as the underlying mechanisms of muscle force production loss (Owens et al., 2019).

An uncontrolled, high influx of extracellular Ca^{2+} into the cell causes further myofibril damage, beginning the process of secondary muscle damage. Disruption to the sarcolemma and sarcoplasmic reticulum and opening of the stretch-activated channels allows extracellular calcium to enter the cell and intracellular muscle proteins to leak out to the circulation (such as CK and myoglobin in plasma) (Baird et al., 2012; Howatson & van Someren, 2008). Calpains, calcium-dependent proteases, are activated and contribute to the degradation of structural and contractile proteins (Thiebaud, 2012). This influx of calcium also serves as a signal for immune cell infiltration, the activity of which further contributes to secondary muscle damage; however, it is necessary for the clearance of cellular debris and the initiation of tissue repair, an essential stage of the pro-inflammatory phase (Tidball & Villalta, 2010). This influx of calcium also serves as a signal for immune cell infiltration, triggering apoptosis pathways which further contributes to secondary muscle damage; however, it is necessary for the clearance of cellular debris and the initiation of tissue repair, an essential stage of the pro-inflammatory phase (Tidball & Villalta, 2010).

2.1.3 Symptoms and their implications

EIMD is characterised by a range of symptoms that can affect athletic performance. Muscle soreness/DOMS (delayed onset muscle soreness) is the most common symptom; others are reduced muscle strength, decreased force and range of motion, disruptions of myofibrillar structures, swelling and stiffness, as well as inflammation (Baumert et al., 2016). The onset of these symptoms can begin immediately following exercise and persist for up to a week (Owens et al., 2019); however, the peak is typically from 24-48 hours (Fatouros & Jamurtas, 2016). The severity and duration of symptoms depend on the damage to the muscle fibres, with more severe damage resulting in more significant performance impairment (Hyldahl & Hubal, 2014). These implications are of particular concern for athletes with busy exercise schedules, as the recovery time required between training sessions may be extended or performance at subsequent events may be diminished.

2.1.4 Measuring exercise-induced muscle damage

Muscle damage can be measured by various direct and indirect means. Histological analysis is a direct measure in which a muscle tissue sample is taken, the damaged fibres are reviewed under microscopy and the infiltration of inflammatory cells is recorded (Paulsen et al., 2012). In theory, histological analysis is a good measure of muscle damage; however, in practice, muscle tissue sampling is demanding on resources and can be unpleasant for participants (Paulsen et al., 2012). Histological analysis can also be unreliable as the tissue sample is small and not representative of the entire muscle, and the invasive method increases muscle inflammation at the site of sampling, which may influence the inflammatory data when repeatedly sampling over time (Malm et al., 2000). Ultrasound, magnetic resonance imaging (MRI) and electron microscopy are non-invasive direct measures. Ultrasound is considered the gold standard in direct measure methods as it can detect the pennation angle as well as fibre damage and oedema (Walker et al., 2004). MRI is capable of detecting alterations in the structure of skeletal muscle as well as the presence of oedema, however, it lacks the resolution to observe changes at the cellular level (Markus et al., 2021). Whereas, electron

microscopy of a biopsy can identify oedema as well as subcellular damage, including Z-band streaming and smearing (Paulsen et al., 2012). An indirect measure assesses markers altered or caused by muscle damage, such as force production, DOMS, range of motion, swelling, and plasma creatine kinase (CK) levels (Baird et al., 2012; Markus et al., 2021). These indirect markers are often used to measure muscle damage; however, aside from force production, they are not always reliable as they may not accurately reflect the damage or correlate well with each other (Clarkson & Hubal, 2002). Numerous studies and reviews consistently indicate a strong association between muscle damage and reduced muscle force production; therefore, it is widely acknowledged as the most valid indirect measure for assessing muscle damage (Fatouros & Jamurtas, 2016; Owens et al., 2019). The ease and reliability of the method to measure muscle force further strengthen its preference as the primary assessment tool (Paulsen et al., 2012). Losses in force are typically considered from 15% to 60% of pre-exercise force and can linger for up to two weeks in severe cases (Owens et al., 2019).

Creatine kinase

Creatine kinase (CK) is an enzyme found primarily in tissues with high energy demands, such as skeletal, cardiac, and smooth muscle. Studies on exercise-induced muscle damage will typically measure the predominant creatine kinase isoform of skeletal muscle, which is CKM. Plasma CK levels rise immediately after EIMD due to eccentric lengthening damaging the membrane, allowing muscle proteins to leak into circulation (Owens et al., 2019). Plasma CK levels appear to depend on the type and intensity of the exercise and muscle mass, with higher levels typically seen after intense, eccentric movements, peaking around 24 to 72 hours post-exercise (Paulsen et al., 2012). However, CK may not be a reliable measure of EIMD as it weakly correlates with muscle function and is more appropriate for confirming the occurrence of tissue damage; there are differing claims as to whether CK can provide a valid indication of the severity of the muscle damage (Owens et al., 2019; Sciorati et al., 2016). A review by Paulsen et al. (2012), noted that CK levels may be a useful measure to differentiate between subjects with mild muscle damage and severe muscle damage (serum CK 1,000 IU/L and > 10,000 IU/L, respectively). Owens et al. (2019), while agreeing that CK can provide additional information about tissue damage, argue that it does not have a consistent temporal relationship with muscle function and therefore, is not a valid marker of severity.

2.2 The Inflammatory Response

The pro-inflammatory phase brings excess fluid and immune cells to the site of injury, which is critical for the clearance of cell debris and preparation of the tissue for regeneration. This phase is activated by the cell debris and cell content leakage from the damaged muscle fibres (Yang & Hu, 2018). Resident mast cells and neutrophils at the injury site are rapidly activated, and degranulation of their pro-inflammatory cytokines triggers the acute inflammatory response (Yang & Hu, 2018). The main immune cells as well as the pro- and anti-inflammatory cytokines are discussed in detail below in the sections *2.2.1 Cells* and *2.2.2 Cytokines*. These cytokines attract peripheral immune cells such as mast cells, T cells, neutrophils, and macrophages to the injury site (Peake et al., 2015; Yang & Hu, 2018). Neutrophils and macrophages phagocytose damaged muscle cells and cellular debris, express cytokines, and produce cytotoxic molecules such as reactive oxygen species (ROS) and nitric oxide (NO) that further degrade muscle tissue, contributing to secondary muscle damage (Owens et al., 2019; Tidball & Villalta, 2010). If this process continues for a prolonged period, there is a risk of damage to the surrounding healthy tissue and delayed muscle recovery (Chazaud, 2016). Muscle

stem cells, often referred to as satellite cells, activate and proliferate during this stage in preparation for tissue repair (Bentzinger et al., 2013). For the regeneration of muscle tissue, the switch to the anti-inflammatory phase must occur in a timely manner.

The anti-inflammatory phase comprises several key processes involving T cells, macrophages, and satellite cells. A subset of T cells mediates the macrophage phenotype shift from pro-inflammatory to anti-inflammatory (Yang & Hu, 2018). The anti-inflammatory macrophages secrete cytokines that help to repress the pro-inflammatory state and growth factors that are involved in tissue regeneration (Chazaud, 2016; Sciorati et al., 2016). Satellite cells differentiate and either fuse to form new myofibres or fuse to existing fibres to repair damage (Chazaud, 2016; Tidball & Villalta, 2010). The myofibres mature, increasing in size and strength until they are indistinguishable from the surrounding undamaged tissue, concluding the muscle regeneration stage (Yang & Hu, 2018).

2.2.1 Cells

Mast cells

Mast cells are a type of immune cell that reside in muscle fibres; they are rapidly activated following muscle injury. Once activated, mast cells degranulate and release proinflammatory cytokines (TNF-α and IL-1) to recruit more mast cells and other immune cells, such as neutrophils and macrophages (Pillon et al., 2013; Yang & Hu, 2018).

Neutrophils

Neutrophils play a pivotal role in the early pro-inflammatory phase of muscle injury, contributing significantly to tissue repair and adaptation. Neutrophils originate from bone marrow and circulate in the bloodstream until recruited to the site of muscle damage; they are recognised as the first immune cells to infiltrate damaged tissue, typically arriving within 2 hours post-injury and peaking between 12 to 24 hours before diminishing after 72 to 96 hours (Kovtun et al., 2018; Tidball, 2017; Yang & Hu, 2018). Once in the muscle tissue, neutrophils are activated by calcium-stimulated proteolysis and heightened intracellular calcium signalling, prompting the release of pro-inflammatory cytokines (Owens et al., 2019). These cytokines are TNF-α, IFN-γ, and IL-1β, and they recruit additional periphery neutrophils and macrophages to the injury site (Yang & Hu, 2018). The neutrophils engage in phagocytosis (regulated by the cytokines), engulfing necrotic myofibres and cell debris (Kovtun et al., 2018). Neutrophils can also produce reactive oxygen species (ROS) molecules through NADPH oxidase-derived mechanisms, which may exacerbate tissue damage and contribute to secondary muscle damage (Owens et al., 2019).

T cells

T cells are a diverse group of leukocytes involved in the pro- and anti-inflammatory response. They can be broadly categorised into CD4+ and CD8+ subtypes. The pro-inflammatory CD8+ T cells are cytotoxic and induce apoptosis in target cells, assisting with clearing damaged tissue (Yang & Hu, 2018). CD8+ T cells secrete inflammatory cytokines, recruit M1 macrophages to the injury site and enhance their phagocytic abilities (Peake et al., 2016). CD4+ T cells coordinate the immune response by secreting cytokines and interacting with other immune cells; a specific subset is the regulatory T cells (Tregs), which demonstrate anti-inflammatory properties (Peake et al., 2016; Yang & Hu, 2018). Tregs increase significantly during the regeneration phase of EIMD; they secrete IL-10 and other cytokines that facilitate the conversion of M1 to M2 macrophages, promote myoblast differentiation and suppress inflammation (Yang & Hu, 2018).

Macrophages

Macrophages are versatile immune cells that play pro- and anti-inflammatory roles post-EIMD. Monocytes generated in the bone marrow undergo differentiation into tissue-resident macrophages once they settle in skeletal muscle (Pillon et al., 2013). Skeletal muscle harbours a small population of these resident macrophages, mainly in the peri- and epimysium surrounding muscle fascicles and the entire muscle (Chazaud, 2016). The roles of macrophages are varied depending on their phenotype. M1 macrophages are involved in the pro-inflammatory response and, upon resolution of this degeneration phase, transition to their M2 anti-inflammatory phenotype for muscle regeneration (Sciorati et al., 2016).

M1 macrophages

The resident macrophages are triggered upon muscle injury to migrate to the site of tissue damage and become classically activated into their M1 phenotype, after which they undertake pro-inflammatory actions. M1 macrophages secrete pro-inflammatory cytokines (IL-1β, IL-6, and TNF- α), which act as signalling molecules that attract more monocytes (and other leukocytes) to the damaged tissue within hours, their levels peaking at 24 hours (Chazaud, 2016; Pillon et al., 2013). This influx of monocytes persists for a limited time, typically lasting a few days, and evidence suggests that blocking the entry pathway for pro-inflammatory macrophages hampers effective muscle regeneration (Chazaud, 2016; Tidball & Villalta, 2010). M1 macrophages produce cytotoxic levels of nitric oxide (NO) through the activity of inducible nitric oxide synthase (iNOS) and phagocytose dead cells and debris (Tidball & Villalta, 2010; Yang & Hu, 2018). They also stimulate satellite cell proliferation, paving the way for tissue repair and regeneration (Peake et al., 2016). Following the peak accumulation of M1 macrophages, the pro-inflammatory state shifts to an anti-inflammatory state as M2 macrophages take over. This transition from the M1 to M2 phenotype is dependent on the local environment, with triggering signals varying from the complete phagocytosis of cell debris to AMP-activated protein kinase-α and cytokine IL-10 (Peake et al., 2016; Pillon et al., 2013).

M2 macrophages

M2 macrophages play a pivotal role in the resolution of the pro-inflammatory state and the regenerative phases of muscle injury. They attenuate the pro-inflammatory response by secreting anti-inflammatory cytokines (such as IL-10) that suppress the pro-inflammatory cytokine production (Tidball & Villalta, 2010). M2 macrophages facilitate tissue repair by producing growth factors such as transforming growth factor-β1 (TGF-β), insulin-like growth factor-1 (IGF I), and vascular endothelial growth factor (VEGF). TGF-β supports tissue repair and modulates the synthesis of connective tissue, IGF I enhances myoblast proliferation, and VEGF contributes to mesenchymal-endothelial cross-talk and facilitates the formation of blood vessels to new muscle tissue (Pillon et al., 2013; Sciorati et al., 2016). M2 macrophages further contribute to the regenerative process by recusing satellite muscle cells from apoptosis and stimulating their differentiation (Peake et al., 2016). The close relationship between M2 macrophages and muscle regeneration is supported by studies in which depletion of the M2 phenotype resulted in a disruption of myoblast differentiation, muscle repair and growth following injury (Pillon et al., 2013; Sciorati et al., 2016).

Satellite cells

Muscle tissue regeneration is facilitated by satellite cells, the skeletal muscle stem cells, which activate and proliferate during the pro-inflammatory phase and differentiate during the anti-inflammatory phase. These cells reside beneath the basal lamina, alongside the muscle fibre, and

remain quiescent until activated. Satellite cells can be activated by strenuous activity or muscle damage due to the endogenous molecules (damage-associated molecular patterns) which are released by damaged or dying cells (Yin et al., 2013). They can also be activated by cytokines IL-6, TNF-α, IL-1, and IGF-1, as well as nitric oxide (Yin et al., 2013). Following activation satellite cells enter the proliferation stage, undergoing rapid cell proliferation, resulting in a large number of myogenic precursor cells (Yin et al., 2013). Once proliferated some satellite cells remain close to the muscle fibre which facilitates the replenishment of the satellite cell pool for future needs, whereas other proliferated satellite cells migrate to the injured muscle tissue for regeneration (Bentzinger et al., 2013). From there the migrated cells begin to differentiate; this process is regulated by specific proteins, such as myogenin, and involves various stages (Tidball & Villalta, 2010). In early differentiation the myoblasts fuse to form myotubes, and myogenin drives the expression of muscle-specific genes such as myosin heavy chain, desmin, and muscle creatine kinase (Tidball & Villalta, 2010). During terminal differentiation the expression of these genes increases to high levels. The myotubes maturate into fully formed, multinucleated muscle cells, from which they either fuse to form new myofibres or fuse to existing muscle fibres to repair damage (Bentzinger et al., 2013; Tidball & Villalta, 2010; Yin et al., 2013).

2.2.2 Cytokines

Cytokines are a group of small proteins produced by various sources, including muscle fibres and leukocytes, that act as intercellular messengers, regulate immune function and influence various other cell processes (Pillon et al., 2013). Exercise-induced muscle contractions and the subsequent muscle damage triggers the release of cytokines; their levels can vary based on the type, intensity, and duration of exercise (Peake et al., 2015). Cytokines are crucial for muscle repair following exercise-induced damage and play pro- and anti-inflammatory roles in the recovery of function and muscle adaptions (Paulsen et al., 2012).

IFN-γ

Interferon-gamma (IFN-γ) is crucial cytokine in the pro-inflammatory immune response. It is primarily secreted in high amounts by T cells, it is also released by resident neutrophils and mast cells (Yang & Hu, 2018). IFN-γ activates macrophages to their pro-inflammatory M1 subtype, enhancing their effectiveness in phagocytosis of muscle debris (Tidball & Villalta, 2010). While local levels of IFN-γ increase post-EIMD, there is no significant increase in the systemic concentrations of this cytokine (Peake et al., 2015). Therefore, it will not be measured as a biomarker in this study.

IL-1B

Interleukin-1β (IL-1β) is primarily produced by macrophages, it is also secreted by T cells, neutrophils, and mast cells. This cytokine recruits additional immune cells to the injury site, and it has also been shown to stimulate the production of interleukin-6 (IL-6) in skeletal muscle cells, suggesting a direct impact on muscle tissue (Yang & Hu, 2018). IL-1β is often evident in the systemic inflammatory response associated with EIMD, with circulatory levels of this cytokine present after extensive EIMD (Fatouros & Jamurtas, 2016). Therefore, it will be used as a biomarker in this study. These levels may remain elevated into the anti-inflammatory phase as IL-1β has been implicated in activating satellite cells, suggesting a role within muscle regeneration (Fatouros & Jamurtas, 2016).

TNF-α

Tumor Necrosis Factor-alpha (TNF-α) is a complex cytokine involved in both pro- and anti-inflammatory responses, playing pivotal roles in inflammation regulation. TNF-α is primarily

produced by activated M1 macrophages, T cells, and neutrophils (Tidball & Villalta, 2010). Skeletal muscle has increased local TNF-α expression after exercise, peaking at 24 hours while circulating concentrations show minimal changes (Peake et al., 2015); therefore, TNF-α will not be used as a biomarker in this study. TNF-α acts as a chemoattractant, recruiting neutrophils and macrophages to damaged tissue and inducing migration of stem cells towards M1 macrophages (Tidball & Villalta, 2010). TNF-α promotes the activation of macrophages to the M1 phenotype and the activation and proliferation of satellite cells (Bentzinger et al., 2013; Tidball & Villalta, 2010). TNF- α also has been shown to influence muscle cells during regeneration by binding directly to these cells via receptors (Peake et al., 2015).

IL-6

IL-6 was initially believed to be a solely pro-inflammatory cytokine; however, it has been found to also exhibit anti-inflammatory actions. IL-6 is released in response to calcium-dependent stimuli during muscle contraction (Pillon et al., 2013), and it has been shown that serum concentration is higher after eccentric movements compared to concentric, levels peaking within a few hours after exercise (Almada et al., 2013; Paulsen et al., 2012). The pro-inflammatory actions of IL-6 include the recruitment of peripheral neutrophils to the injury site and stimulation of muscle cells to release chemical signaling molecules that attract peripheral monocytes and T cells (Scheller et al., 2011). The monocytes differentiate into M1 macrophages and, alongside the other recruited leukocytes, secrete IL-6 (Pillon et al., 2013; Scheller et al., 2011). IL-6 also activates NADPH oxidase in neutrophils and macrophages; this is an enzyme complex that is involved in the release of reactive oxygen species (ROS), essential for enhancing the phagocytic activity of these leukocytes (Fatouros & Jamurtas, 2016; Scheller et al., 2011). Once the muscle cell debris has been cleared and the shift towards the anti-inflammatory phase begins, IL-6 is involved in the clearance of neutrophils, binding via a receptor and inducing apoptosis of the neutrophils (Scheller et al., 2011).

Il-6 levels decrease after the resolution of the pro-inflammatory phase; however, they remain elevated above baseline for a few days after, playing a role in muscle regeneration. IL-6 triggers T cells to release the anti-inflammatory cytokine IL-10 and inhibits macrophage production of the pro-inflammatory cytokines TNF-α and IL-1β (Fatouros & Jamurtas, 2016; Kistner et al., 2022). IL-6 is involved in the activation, proliferation, and differentiation of muscle satellite cells and, therefore, the formation of myotubes (Fatouros & Jamurtas, 2016; Pillon et al., 2013; Yang & Hu, 2018). In 1L-6 null mice, there was a loss of normal muscle cell differentiation and slowed muscle growth; this suggests that the loss of IL-6 signaling impairs the transition from proliferation to the differential stage of muscle regeneration (Tidball & Villalta, 2010).

IL-10

IL-10 is an anti-inflammatory cytokine involved in immune regulation and tissue regeneration. IL-10 local expression increases following exercise; however, changes in plasma concentrations are variable between studies and participants (Paulsen et al., 2012; Peake et al., 2015). IL-10 is initially secreted by T cells (Tregs), alongside other cytokines, to facilitate the conversion of M1 to M2 macrophages (Yang & Hu, 2018). Once activated, M2 macrophages also secrete high levels of IL-10 (Peake et al., 2016). Facilitating this shift in macrophage phenotype increases myoblast proliferation and differentiation, encouraging muscle regeneration and growth (Peake et al., 2016; Pillon et al., 2013). IL-10 also suppresses the pro-inflammatory state by deactivating M1 macrophages and inhibiting cytokine IL-1β, which induces IL-6 secretion and inhibits myogenin (Peake et al., 2016).

2.2.3 Modulating the inflammatory response

Exercise-induced muscle damage often leads to delayed onset muscle soreness (DOMS), impacting performance, recovery, and time between training sessions. This has prompted investigations into mitigating its effects on athletes and active individuals. Historically, DOMS was widely associated with the pro-inflammatory phase of the inflammatory response, due to which significant research has explored modulating the pro-inflammatory response. Although it is becoming increasingly evident that DOMS is a complex phenomenon and is not solely caused by inflammation; it also involves the activation of Type III and IV nerve fibres, calcium dysregulation, and the release of chemical mediators such as prostaglandins and histamine (Urso, 2013).

The initial pro-inflammatory response to clear cell debris and activate satellite cells is crucial for muscle tissue regeneration. Neutrophils and M1 macrophages clear dead cells and debris through phagocytosis and reactive oxygen species (ROS) production. ROS is an important signalling molecule for muscle adaptation; however, excessive ROS production can exacerbate muscle damage, affecting healthy tissue and contributing to fibrosis (Chazaud, 2016; Peake et al., 2016). Fibrosis occurs when dead cells are not adequately removed and connective tissue is excessively deposited, leading to maladaptive muscle remodelling with collagen and ectopic fat forming scar tissue (Sciorati et al., 2016). Therefore, the interactions between leukocytes and muscle tissue must be regulated to avoid prolonged inflammation. This supports the desirability of modulating the pro-inflammatory stage; however, excessive suppression can compromise muscle regeneration (Fatouros & Jamurtas, 2016; Peake et al., 2016).

Interventions to suppress the pro-inflammatory state, such as non-steroidal anti-inflammatory drugs (NSAIDs), have shown inconsistent findings and may negatively affect muscle regeneration (Howatson & van Someren, 2008; Urso, 2013). While commonly used to reduce pain and inflammation, NSAIDs may hinder muscle recovery by suppressing satellite cell activity, impacting protein synthesis, and interfering with mitochondrial adaptations to exercise (Urso, 2013). In a study with 8 healthy, recreationally trained males (23 ± 3 years old), NSAID infusion decreased satellite cell number and activity but did not change inflammatory markers (Mackey et al., 2007). Participants performed 200 maximal eccentric leg contractions, then rested for 4.5 hours with either NSAIDs or a placebo infused directly to the vastus lateralis muscle. The number of satellite cells in the placebo group increased by 96% by day 8 after exercise ($P = < 0.05$); while the NSAIDs group showed no increase. Since satellite cells drive muscle regeneration post-exercise, NSAID-induced blockage of cell activity has negative implications for muscle recovery and adaptations.

Two studies demonstrated conflicting findings on muscle damage markers with NSAIDs administration. Donnelly et al. (1990) found no difference in soreness, strength or endurance; however, muscle damage markers were higher in the NSAID group. Thirty-two healthy males (21 ± 1 years old) performed downhill running and consumed either 600 mg ibuprofen or a placebo 30 minutes prior to the exercise, and every 6 hours until 72 hours post-exercise, for a total dose of 8400 mg. The NSAIDs group had higher concentrations of plasma CK and urea ($P = < 0.01$) compared to the control group. Conversely, another study found reduced markers of muscle damage with NSAID administration (Rahnama et al., 2005). Twenty-two healthy, non-athletic male participants performed 70 eccentric arm curls at 80% maximal voluntary contraction, and the NSAIDs group received 400 mg ibuprofen 1 hour prior to exercise and every 7 hours until 48 hours post-exercise, for a total dose of 2800 mg. There was no difference in range of movement or strength; however,

the NSAIDs group had lower serum CK and lower perceived muscle soreness (both P= < 0.01) compared to the control group. These contradictory findings may be related to the difference in NSAIDs dosage, with the three times higher dose in the Donnelly et al. (1990) study showing increased muscle damage compared to the lower dose in the Rahnama et al. (2005) study, which demonstrated reduced muscle damage.

While acute NSAIDs administration in young, healthy adults have inconsistent effects, chronic administration in older adults may positively influence exercise adaptation. In a study by (Trappe et al., 2010), 36 healthy male and female adults (65 ± 1 years old) performed lower body resistance training 3 days a week for 12 weeks and consumed either a placebo or 400 mg NSAIDs three times daily (1200 mg total). Muscle mass and strength increased more in the NSAIDs group than the placebo group (P= < 0.05). This finding is attributed to the suppression of the low-grade systemic inflammation associated with aging, which has been associated with lower skeletal muscle strength and mass (Trappe et al., 2010; Tuttle et al., 2020). Occasional NSAID use post-exercise may not be problematic, but its efficacy in alleviating muscle pain or damage is not validated. However, in cases of chronic inflammation affecting protein turnover, such as in the elderly or those with muscle-wasting conditions, NSAIDs might be beneficial. Regardless, these findings indicate that disturbing the pro-inflammatory phase may hamper the anti-inflammatory phase, affecting muscle tissue regeneration; therefore, modulating the inflammatory process remains a complex challenge.

Anti-inflammatory drugs show inconsistent benefits; however, other strategies may reduce inflammation. Cold water immersion is a popular recovery strategy, and immersion immediately after exercise and repeated throughout the recovery process has demonstrated beneficial effects on maximal strength, countermovement jump, reduced muscle soreness, reduced local swelling and decreased CK and myoglobin concentrations (Nédélec et al., 2013). Swelling transports immune cells such as leukocytes and monocytes to the injured tissue (Wilcock et al., 2006). Cold temperature induces a redirection of blood flow from the limbs to the core, reducing limb swelling and potentially reducing immune cell infiltration and secondary muscle damage (Nédélec et al., 2013; Wilcock et al., 2006). However, regular use of this strategy may hinder exercise adaptations by interfering with the acute inflammatory process (Nédélec et al., 2013).

Adequate sleep is crucial for muscle recovery and good health. Partial sleep deprivation has been shown to alter the monocyte pro-inflammatory response and the expression of these genes (Nédélec et al., 2013). Even one night of sleep deprivation (awake from 11pm to 3am) can exacerbate the pro-inflammatory response compared to uninterrupted sleep (Irwin et al., 2006). Evidence for other strategies such as active recovery, stretching, massage and compression garments is currently lacking. However, one extensively investigated strategy is the effect of nutrition on inflammation, muscle recovery, and adaptation, which will be explored in detail below.

2.3 Nutrient Effects on Inflammation

Nutrition plays a pivotal role in modulating inflammation and facilitating recovery from EIMD. Nutrients such as proteins, fats, carbohydrates, antioxidants, and minerals are essential in these processes. Proteins are vital for muscle repair and growth (Arentson-Lantz & Kilroe, 2021), and some dietary fats can exhibit anti-inflammatory properties (Owens et al., 2019). Antioxidants play a role in mitigating oxidative stress and inflammation, although their effects on EIMD recovery remain debated (Peake et al., 2007). The mineral zinc modulates immune function and inflammatory responses, with deficiency impairing muscle function and hindering recovery (Gammoh & Rink, 2017; Hernández-Camacho et al., 2020). The influence of these nutrients and their potential benefits in optimising post-exercise recovery are explored in more detail below.

2.3.1 Protein

Proteins are composed of amino acids and are vital for muscle repair, growth, and overall function. Skeletal muscle mass is regulated by muscle protein synthesis (MPS) and breakdown (MPB), which is influenced significantly by physical activity and dietary protein intake (Arentson-Lantz & Kilroe, 2021; Kerksick et al., 2021; Pinckaers et al., 2022). Resistance and endurance exercises stimulate MPS but also increase protein breakdown; therefore, protein intake post-exercise is crucial for muscle repair and growth (Churchward-Venne et al., 2020; Pasiakos et al., 2014). Dietary protein acts as an anabolic stimulus and a source of essential amino acids necessary for MPS (Arentson-Lantz & Kilroe, 2021; Pinckaers, Smeets, et al., 2024). The body needs to catabolise its protein stores if it does not receive adequate dietary protein to meet essential protein requirements (Kreider & Campbell, 2009; Levenhagen et al., 2002).

Dietary proteins are classed as complete or incomplete based on their amino acid profile. Complete proteins are found primarily in animal-based sources and provide all the essential amino acids necessary for bodily functions (Kerksick et al., 2021; Kreider & Campbell, 2009). Animal protein sources include meat, poultry, fish, eggs, and dairy products (Kreider & Campbell, 2009). Plant-based proteins are considered incomplete as they lack some essential amino acids. Plant protein sources include legumes, nuts, seeds, grains, and soy products such as tofu (Kerksick et al., 2021).

Dietary protein has been extensively researched in relation to its potential influence on recovery from EIMD and inflammation. Protein is crucial for muscle fibre repair and adaptation due to its amino acid content; however, its role in reducing inflammation may be more closely associated with co-ingestion of phytochemicals, particularly in plant-based proteins (Hruby & Jacques, 2019). Studies investigating inadequate protein intake have reported adverse outcomes, confirming the importance of meeting protein needs to ensure optimal muscle repair and recovery (Pasiakos et al., 2014).

There are differences in amino acid composition and digestibility between plant and animal-based proteins, which affects the rate of MPS. Animal-based proteins are typically well-digested and have a complete amino acid profile high in branched-chain amino acids (Arentson-Lantz & Kilroe, 2021). Whereas plant-based proteins often have lower digestibility and may lack specific essential amino acids such as lysine and methionine (Arentson-Lantz & Kilroe, 2021; Kerksick et al., 2021; Pinckaers, Smeets, et al., 2024). Plant-based proteins may also be more readily converted to urea, reducing the available amino acids for MPS compared to animal-based proteins (van Vliet et al., 2015). These factors can limit plant proteins' ability to stimulate MPS, attenuate MPB, and promote muscle recovery post-exercise (Pinckaers, Domić, et al., 2024; van Vliet et al., 2015). However, plant protein

supplements can be purified to increase their digestibility comparable to animal-based proteins (Pinckaers et al., 2021; van Vliet et al., 2015). Studies comparing the effects of plant-based proteins, such as pea or soy protein, with animal-based proteins like whey have shown that both can mitigate muscle damage post-exercise and increase muscle mass; however, whey protein typically tends to have a more pronounced effect, likely due to its high leucine content and higher digestibility (Kritikos et al., 2021; Nieman et al., 2020; Pinckaers, Domić, et al., 2024; Wilkinson et al., 2007; Xia et al., 2018).

The amino acids leucine and glutamine influence MPS and modulate inflammation. Leucine maximally stimulates MPS by activating the mTORC1 pathway, which results in the upregulation of MPS (Arentson-Lantz & Kilroe, 2021). Leucine has been shown to enhance recovery from EIMD and protect against muscle loss during periods of inactivity (Arentson-Lantz & Kilroe, 2021; Howatson et al., 2012). Leucine is able to counteract the catabolic effects of the pro-inflammatory phase by enhancing protein translation initiation and attenuating proteolysis (Nicastro et al., 2012). Unlike leucine, the body can synthesise glutamine; however, after intense exercise or trauma, the demand for glutamine may exceed the body's ability to produce it (Nicastro et al., 2012). Glutamine plays an essential role in the function of many leukocytes, including influencing apoptosis and cytokine production, such as IL-10 and IFN-γ (Nicastro et al., 2012).

Proteins are essential for muscle repair and growth as their intake affects MPS and MPB. Animal and plant-based proteins offer differing advantages depending on their digestibility and amino acid content. Understanding the nuances of dietary protein intake is important for optimising muscle recovery post-EIMD.

2.3.2 Carbohydrates

Carbohydrates play a critical role in exercise performance and muscle recovery. During high-intensity exercise, carbohydrates are the primary energy source for skeletal muscle, delaying fatigue and improving performance by maintaining muscle and liver glycogen stores (Ivy, 2004). Post-exercise carbohydrate intake is needed to replenish glycogen stores, promote recovery and reduce muscle damage. A study conducted by Roy et al. (1997) found that carbohydrate supplementation post-resistance exercise increased protein synthesis by 36% and reduced markers of muscle tissue damage and protein degradation. This is attributed to ingestion of carbohydrate triggering insulin release. Insulin increases muscle amino acid uptake and protein synthesis in skeletal muscle (Ivy, 2004). In a study comparing carbohydrate vs. placebo beverages post-eccentric exercise, it was found that serum insulin was four times higher in the carbohydrate group compared to placebo (Wojcik et al., 2001).

The impact of dietary-sourced carbohydrates on general well-being is well understood. Carbohydrates are classified as simple or complex (Thompson, 2017). Dietary fibre is a complex carbohydrate and high intakes are associated with lower levels of systemic inflammation and lower risk of inflammatory diseases such as type 2 diabetes and cardiovascular disease (Buyken & Brand-Miller, 2013). Simple carbohydrates, such as glucose and fructose, are an important energy source; however, excessive intake is associated with negative health outcomes such as diabetes, obesity, high blood pressure, cardiovascular disease and raised systemic inflammation (Banik et al., 2020; Preuss & Bagchi, 2020; Thompson, 2017).

The influence of carbohydrate (CHO) supplementation on exercise-induced inflammation is less clear, however, dose timing appears to be a factor. Two studies investigating pre- and during-exercise CHO supplementation reported beneficial effects. In a study with 30 marathon runners, CHO supplementation attenuated the post-exercise inflammatory response (Nehlsen-Cannarella et al., 1997). Participants consumed a 6% CHO or placebo beverage every 15 minutes during a 2.5 hour high-intensity treadmill run. The CHO group had lower levels of IL-6, IL-1ra, and cortisol compared to the placebo group. Another study with 8 male runners also found CHO supplementation reduced inflammatory markers (Chen et al., 2008). Participants consumed either a high 65% CHO meal (H-H) or a low 36% CHO meal (H-L) prior to running at 70-76 % VO_{2max} for 2 hours. Post-exercise, the high CHO group had lower levels of IL-6 and IL-10; but there was no difference in cortisol levels.

Conversely, no difference in inflammatory markers were found in two studies with post-exercise CHO supplementation. In a cross-over study by Miles et al. (2007), 8 non-trained participants performed high-force eccentric elbow-flexion exercises and then consumed either a placebo or 0.25 g/kg CHO per hour for hours 0-12 and 24-36 post-exercise. There was no difference in IL-6, CRP, cortisol or CK between the groups. Another cross-over study of a simulated rugby 7s tournament had similar findings (Fabre et al., 2022). Twelve professional male rugby players participated in three rugby 7s tournament days with a week's interval between, each day had three matches with 2 hours recovery between. Participants were required to drink either a placebo or an 80 g CHO drink at the end of each match. The CHO drink improved performance, attenuated CK increase, reduced DOMS and reduced muscle soreness compared to the placebo; however, there was no difference in inflammatory markers IL-6, TNF-α and IL-1ra.

Research into the effect of carbohydrate sources from habitual diets (instead of supplementation) on EIMD and exercise-induced inflammation is lacking. Vegan diets typically feature higher carbohydrate consumption due to the emphasis on fruits, vegetables, whole grains, and legumes (Craddock et al., 2016). This increased carbohydrate intake may lead to higher muscle glycogen concentrations, which can improve endurance performance and delay fatigue (Craddock et al., 2020). However, further research is needed to determine whether this benefit also applies to muscle recovery and inflammation post-exercise.

2.3.3 Fat

Dietary fat intake has a complex relationship with inflammation as it impacts various metabolic processes and contributes to the development of metabolic disorders. Excessive consumption of saturated fats, particularly from animal sources such as red meat and dairy, has been associated with systemic inflammation and insulin resistance (Franco-de-Moraes et al., 2017). High-fat intake can increase the presence of innate immune cells like macrophages in skeletal muscle (Pillon et al., 2013). Saturated fatty acids drive this inflammatory response through cross-talk between muscle and immune cells (Pillon et al., 2013). When exposed to saturated fatty acids, macrophages and muscle cells release inflammatory cytokines, which further perpetuate inflammation within the tissue (Pillon et al., 2013). Conversely, unsaturated fats exhibit anti-inflammatory properties and are found in nuts and oily, wild-caught fish such as tuna and salmon (Franco-de-Moraes et al., 2017). Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been shown to reduce systemic inflammation and oxidative stress induced by damaging exercise (Owens et al., 2019).

Fish oil supplementation reduced oxidative stress markers after eccentric exercise in a study by Gray et al. (2014). Twenty healthy, recreationally active males (20-25 years old) performed 200 eccentric

knee contractions after 6 weeks of daily supplementation with 3 g fish oil. There was no difference in plasma CK, muscle soreness or maximal voluntary contraction; however, the fish oil group had lower oxidative damage to lymphocytes and lower lipid peroxidation (Gray et al., 2014). A strength of this study was that the control group was not put on a n-3 PUFA-deficient diet; participants were allowed to maintain their habitual diets. However, aside from recording weekly consumption of n-3 PUFA in both groups, a comprehensive nutritional analysis was not conducted. Therefore, it is unclear whether differences in the intake of other important muscle recovery and antioxidant nutrients may have confounded the results.

Exercise-induced inflammatory markers were ameliorated by fish oil supplementation in a study with 45 healthy, untrained men (20-35 years old) (Tartibian et al., 2011). Participants received daily 1.8 g fish oil supplementation for 30 days before undertaking an eccentric bench stepping exercise for 40 minutes. There was a significant post-exercise decrease in plasma TNF-α, IL-6 and CK in the fish oil group compared to the control group. A strength of this study was the fish oil dose being similar to the recommended daily intake of 1.6 g (National Health and Medical Research Council et al., 2006); making the findings more applicable to the general population compared to other super-supplementation studies.

2.3.4 Antioxidants

Antioxidants play a crucial role in mitigating inflammation and oxidative stress. They can act as defence mechanisms against free radicals and other ROS generated from EIMD (Bell et al., 2015). Excessive ROS can exacerbate muscle injuries by damaging cells; antioxidants might mitigate these effects by neutralising these reactive molecules (Peake, Nosaka, et al., 2005). Antioxidants are broadly classified into various subtypes, such as polyphenols, vitamins, and minerals; they are found in diverse dietary sources (Howatson et al., 2010; Owens et al., 2019).

Phytochemicals

Phytochemicals are naturally occurring compounds found in plants, and they have been the focus of recent research for their potential role in attenuating post-exercise inflammation. Phytochemicals include polyphenols (including phenolic acids and flavonoids), nitrate, carotenoids, and betalains.

Supplementation with polyphenols has demonstrated anti-oxidative and anti-inflammatory properties, contributing to faster muscle recovery post-EIMD. In a study on tart cherry juice administration in marathon running, the treatment group experienced quicker muscle strength recovery and lower inflammatory levels (Howatson et al., 2010). Twenty recreational marathon runners of mixed genders were randomly assigned to a cherry juice or placebo group. Participants were given the beverage for 5 days before, on the day of, and 48 hours after completing a marathon. Findings revealed a faster isometric knee extension strength recovery in the cherry juice group. While markers of muscle damage (creatine kinase, lactate dehydrogenase, muscle soreness) showed no significant differences, the cherry juice group exhibited substantial reductions in inflammatory markers (IL-6, CRP, uric acid). The cherry juice group showed an increase in total antioxidant capacity and a decrease in lipid peroxidation.

A study investigating the impact of Montmorency cherry (MC) supplementation on recovery and performance focused on inflammatory and oxidative stress responses (Bell et al., 2015). MCs are rich in anthocyanins, a subset of polyphenols, which have been shown in previous studies to exhibit effects comparable to NSAIDs in mitigating oxidative stress and inflammatory mediators (Seeram et

al., 2001; Wang et al., 1999). Endurance-trained cyclists were recruited to participate in a cycling exercise model and consume either MC or a placebo for 4 days before the trial until 3 days after. The findings indicated that MC supplementation preserved muscle function across the 72-hour post-exercise period, whereas the placebo group experienced a decline. Inflammatory markers IL-6 and hsCRP were attenuated in the MC group prior to and post-exercise, suggesting a dampened pro-inflammatory response. Contrary to the researcher's hypothesis, the oxidative stress response did not differ. It is important to note that the MC and control groups were instructed to follow a low-polyphenolic diet during the trial; therefore, it is unclear whether the differences were due to MC supplementation or the control group being nutrient-deficient. Future studies that maintain habitual diets and add supplementation on top would be valuable to clarify these findings.

The potential protective effects of beetroot juice against muscle damage post-exercise were investigated by Clifford et al. (2016). Beetroot juice is rich in phenolic acids, flavonoids, carotenoids, nitrate anions and betalains (Clifford et al., 2016). Dietary nitrate can be converted to nitric oxide (NO), and numerous studies propose that NO may exert anti-inflammatory effects by limiting leukocyte activation and reducing pro-inflammatory mediators within cells (Jädert et al., 2012; Jädert et al., 2014). Betalains demonstrate antioxidant and anti-inflammatory properties by inhibiting pro-inflammatory activators, scavenging ROS and upregulating endogenous antioxidant enzymes (Clifford et al., 2016). The study recruited 30 recreationally active males who were randomly assigned to three groups: high-dose beetroot juice (H-BT; 250 ml), lower-dose beetroot juice (L-BT; 125 ml), or an isocaloric placebo (PLA; 250 ml). Participants consumed the supplement immediately, 24, and 48 hours after completing 100-drop jumps. Various measures were assessed, including maximal isometric voluntary contractions (MIVC), countermovement jumps (CMJ), pressure pain threshold (PPT), CK, IL-6, IL-8, and TNF-α at different time points post-exercise. The study found that participants who received high-dose beetroot supplementation (H-BT) showed a faster recovery in their CMJ performance at 48 and 72 hours after exercise. The H-BT and low-dose beetroot supplementation (L-BT) groups experienced reduced muscle soreness compared to the placebo (PLA) group at 24, 48, and 72 hours after exercise. However, other indicators of functional recovery, muscle damage, and inflammation remained unaffected by beetroot supplementation. It is worth noting that, like the Montmorency cherry study (Bell et al., 2015), participants followed a diet low in phenolics and nitrates. Therefore, it is unclear how the nutrient-deficient diet affected the results when compared to the L-BT and H-BT groups.

These studies emphasise the potential anti-inflammatory effects of phytochemical-rich supplements in mitigating exercise-induced muscle damage. These findings suggest that these compounds contribute to faster muscle recovery and reduced inflammatory markers. However, it is important to note the dietary restrictions imposed on participants in two studies, which may have influenced the results. While these studies highlight the promising role of phytochemicals in muscle recovery, further research under diverse dietary conditions is needed for a comprehensive understanding of their effects.

Vitamins

The effects of vitamin supplementation on EIMD and inflammation are a contested topic. Research findings regarding the efficacy of antioxidant supplementation, particularly vitamins C and E, are equivocal. While some studies suggest a positive effect on protecting against oxidative stress and muscle damage, others provide evidence for either no effect or even negative consequences of

antioxidant supplementation (McGinley et al., 2009; McLeay et al., 2012; Owens et al., 2019; Peake et al., 2007; Urso, 2013). These contradictory findings are thought to be attributed to the wide variations in study designs: population studied (trained vs untrained), supplementation protocol (synthetic vs naturally occurring, and dosing), duration of trial, and exercise type performed (upper vs lower body) (McGinley et al., 2009; Peake et al., 2007). Of concern are the studies that reported attenuated inflammatory responses and delayed EIMD recovery with high-dose supplementation; it is theorised that this is caused by interference with cellular signalling pathways and muscle adaptations (Clifford et al., 2016; Peake et al., 2007; Urso, 2013).

Interestingly, emerging evidence suggests that vitamin D may help in muscle regeneration and remodelling. Studies have shown associations between vitamin D levels and muscle function, immune responses, and muscle soreness; however, the exact mechanisms and optimal supplementation strategies for EIMD require further investigation (Owens et al., 2019). Considering the equivocal findings for vitamins C and E, high dosing concerns, and questions regarding vitamin D mechanisms, further research is needed before supplementation can be safely recommended for EIMD.

Zinc

Zinc is an essential nutrient due to its abundance in the human body and its various physiological functions. It is primarily distributed in skeletal muscle and bone, and since the human body does not have a zinc storage system, daily intake is necessary (Gammoh & Rink, 2017; Hernández-Camacho et al., 2020). Zinc is found in high concentrations in red meat, certain seafood, whole grains, and legumes (Thompson, 2017). The bioavailability of zinc from food sources varies, with the highest being from animal products; raising concerns that a vegan diet may not provide sufficient dietary zinc (Gammoh & Rink, 2017; Thompson, 2017).

Given the necessity of daily zinc intake, understanding its physiological roles is crucial. Zinc acts as a cofactor for enzymes and modulates metabolic pathways, influencing phagocytosis, chemotaxis, cytokine production, and combating oxidative stress and inflammation (Gammoh & Rink, 2017; Hernández-Camacho et al., 2020). It modulates pro-inflammatory cytokine production by inhibiting NF-κB activation (Gammoh & Rink, 2017; Hernández-Camacho et al., 2020). Zinc exhibits anti-oxidative properties by activating the Nrf2 transcription factor, which upregulates the antioxidant system and inhibits the production of reactive oxygen and nitrogen species (Hernández-Camacho et al., 2020; Maywald et al., 2017). Zinc is also a cofactor for superoxide dismutase, a major anti-oxidative enzyme (Andrade & Marreiro, 2011). Zinc is crucial for proteostasis, the balance between protein synthesis and degradation, by regulating key pathways such as autophagy and the ubiquitin-proteasome system (Hernández-Camacho et al., 2020). Recent research suggests that zinc is involved in myogenesis, promoting satellite cell proliferation, activation, differentiation, and maturation (Hernández-Camacho et al., 2020). Zinc deficiency is linked to several health conditions. Even mild deficiencies can harm immunity, impair wound healing, and cause low-grade cytokine production and increased oxidative stress (Maywald et al., 2017). Zinc deficiency can impair thymic function, reduce T-cell counts, and hinder antibody production (Gammoh & Rink, 2017; Maywald et al., 2017). Maintaining adequate zinc intake is essential for optimal nutrition, good immunity, and regulation of inflammatory responses.

There is limited research available on the relationship between zinc and skeletal muscle recovery post-exercise; however, its effects on performance and antioxidant status have been explored. In a

study on zinc depletion on exercise performance with 8 healthy male participants (20-35 years old), endurance in knee and shoulder extensor muscles declined significantly (Van Loan et al., 1999). Participants performed knee and shoulder extension exercises on two occasions: once after following a zinc-deficient diet for 33 or 40 days, and again after following a zinc-repletion diet for 27 or 34 days. Peak force was not affected by zinc deficiency; however, exercise endurance for knee and shoulder extensor muscles declined significantly ($P = < 0.01$) and did not recover post the zinc-repletion period.

In a study on the effects of zinc supplementation on the antioxidant status of wrestlers and sedentary participants, it was found that the antioxidant system was activated in participants receiving supplementation. Forty male participants (15-17 years old) were assigned one of four groups; wrestler with or without supplementation, or sedentary with or without supplementation. Zinc supplementation was given daily for 8 weeks, during which the wrestlers continued their usual training sessions of 4-6 days per week. At the end of the 8-week period, it was found that compared to the non-supplemented group, both the sedentary group and the wrestling group that received zinc supplementation had higher serum concentrations of glutathione and zinc, and higher levels of glutathione peroxidase and superoxidase dismutase activity ($P = < 0.01$).

Overall, these findings highlight the importance of zinc in supporting the immune and inflammatory response and maintaining overall health. While research on the relationship between zinc and muscle recovery post-exercise is lacking, the current evidence underscores the necessity of adequate zinc intake; therefore, it can be speculated that zinc deficiency could impair muscle recovery. Future research should explore the potential impacts of zinc supplementation on muscle recovery to better understand its benefits and mechanisms.

2.3.5 Supplementation vs. whole food sources

Nutrient supplementation and whole food sources can play roles in muscle recovery and inflammation post-exercise; however, it is crucial to differentiate between the two. Many studies focus on nutrient supplementation, which often lead to varying results compared to nutrients derived from whole food sources. Although a vegan diet is typically higher in anti-oxidative nutrients, this benefit may be offset by the high fibre content as fibre can lower polyphenol bioavailability, potentially leading to increased oxidative stress markers (Liu et al., 2024; Vanacore et al., 2018). A significant portion of antioxidant compounds are also fermented by the gut with less than 5% reaching the blood stream, this reduced bioavailability is often insufficient to confer any noticeable muscle recovery benefits (Clifford, 2004). Thus, reduced bioavailability and absorption rates highlight the challenges of achieving therapeutic levels of nutrients through diet.

The effects of habitual diets on muscle recovery and inflammation are not well understood, as research often emphasizes supplementation over the overall quality and quantity of diet. Future studies should aim to explore these dietary patterns to provide clearer insights. While whole plant foods may have potential anti-inflammatory benefits, the real-world impact on EIMD and overall recovery remains ambiguous without more comprehensive research. This underscores the need for a balanced approach that considers both nutrient supplementation and whole food sources to optimize muscle recovery and manage inflammation effectively.

2.4 Vegan and Omnivorous Diets

2.4.1 Dietary characteristics and health implications

Vegan and omnivore diets have distinct dietary patterns, leading to differing micro- and macronutrient intake. Vegan diets exclude all animal derived products, including meat, diary, eggs, and sometimes honey, relying primarily on plant-based foods such as vegetables, fruit, grains, legumes, nuts, and seeds (Craddock et al., 2016; Eichelmann et al., 2016). Whereas, omnivorous diets incorporate a variety of foods, including both plant and animal-derived products (Allen et al., 2003). Vegan diets tend to be lower in saturated fat and cholesterol, while typically higher in dietary fibre, carbohydrates, antioxidants, and phytochemicals (Haddad et al., 1999; Menzel, Biemann, et al., 2020). However, they are at risk of inadequate intake of certain nutrients like vitamin B12 and D, iron, calcium, zinc, omega-3 fatty acids, and protein (Craig, 2009; Thompson, 2017). Omnivorous diets typically provide these nutrients, and often higher saturated fat, but may lack folic acid, magnesium, vitamins C, E, and niacin (Appleby & Key, 2016; Craig, 2009; Wegmüller et al., 2017).

Both dietary patterns have been associated with various health outcomes. Vegan diets are associated with a lower BMI (Dinu et al., 2017), this body composition can be advantageous in certain sports (Barnard et al., 2019) and can be protective against dietary related diseases (Menzel, Biemann, et al., 2020). Lower total energy intake is one of the causes thought to be attributable to a lower BMI; however, insufficient energy intake can have detrimental effects on performance including delayed recovery (Holtzman & Ackerman, 2019; Loucks, 2004). Vegans typically have a lower risk of metabolic syndrome, type 2 diabetes, and cardiovascular disease, likely due to their lower saturated fat intake, lower BMI, and higher fibre intake (Kahleova et al., 2018; Lee & Park, 2017). Conversely, omnivores are at higher risk of cardiovascular disease and other inflammatory diseases, as diets high in animal proteins often contain high levels of saturated fat, which can induce inflammation and impair muscle recovery when frequently consumed in excess (Calder, 2015; Hu, 2003; Lowery, 2004). However, protein from animal sources is more bioavailable than plant-based (Michael & Jay, 2004) and is a great source of all essential amino acids, creatine, vitamin D and B12, omega-3, and zinc; nutrients that are pivotal for muscle recovery and adaptations (Craddock et al., 2023; Craig, 2010). Vitamin B12 deficiency is common among vegans, which can result in reduced production of red blood cells, leukocytes, and platelets, potentially impacting the immune response (Haddad et al., 1999). Overall, while vegan diets offer certain health benefits, concerns remain regarding adequate nutrient intake and immune status, highlighting the importance of careful nutritional planning. Whereas omnivore diets may pose risks for cardiovascular health but offer advantages for muscle function and recovery.

2.4.2 Inflammatory differences

Understanding the inflammatory profile of vegans and omnivores provides additional insight into the dietary influence on physiological functions, and comparing systemic inflammation is a crucial first step. Plant-based diets have been shown to reduce chronic inflammation when compared to omnivorous diets. Studies examining systemic inflammation found an overall improvement in inflammatory profiles (lower: CRP, IL-6, fibrinogen, and total leukocytes) in participants who follow vegetarian diets compared to omnivorous (see Table 2.1) (Craddock et al., 2019; Eichelmann et al., 2016; Franco-de-Moraes et al., 2017; Haddad et al., 1999). These studies reviewed a varied population of participants, from healthy and young to those with comorbidities, and in some instances, the intervention was to prescribe a vegetable dominant diet to habitual omnivores (Eichelmann et al., 2016). It should be noted that the vegetarian diets included those who ate eggs

and dairy products, few were strictly vegan. Conversely, no significant differences were found in two studies comparing CRP, TNF-α, IL-6 IL-18, IL-1, RA, ICAM-1, adiponectin, omentin-1 and resistin in vegans and omnivores (Jenko Pražnikar et al., 2023; Menzel, Biemann, et al., 2020), see Table 2.1. However, the researchers of one study noted that their findings may have been influenced by an insufficient sample size as it was powered for the primary research question of differences in bone health between vegans and omnivores (Menzel, Biemann, et al., 2020). The majority of research on systemic inflammation agrees that inflammatory markers are attenuated in plant-based eating patterns. Raised levels of circulating inflammatory biomarkers are associated with lower skeletal muscle mass and strength (Tuttle et al., 2020); and while omnivores may have inflammation within the normal reference ranges, it is yet to be determined whether vegans have an enhanced muscle recovery time due to lower levels of systemic inflammation.

The physiological response of omnivores post-exercise has been well-investigated, however, there is limited research into whether this response is similar in habitual vegans. The research that has been conducted, appears to focus on performance differences, and strength and muscle mass adaptations, as opposed to inflammation. Very little research has explored habitual vegan dietary patterns, often the dietary intervention is to prescribe a vegetarian or vegan diet to habitual omnivores, see Table 2.1 (Richter et al., 1991). This method increases the risk of unknown variables, such as differences in long-term nutritional stores or body composition. One study acknowledged this gap and investigated inflammation post-endurance exercise in habitual diets, they found that there was no differences in immune or inflammatory biomarkers; however, total red blood cell count, haemoglobin and haematocrit levels were lower in the vegan dietary group, see Table 2.1 (Craddock et al., 2023).

As EIMD is a form of tissue wound, it is worth considering studies that have investigated the inflammatory and healing differences between habitual vegans and omnivores in skin wounds. One study comparing post-surgical scars found that vegans had worse outcomes than omnivores, see Table 2.1 (Fusano et al., 2020). This difference was attributed to low pre-surgery serum levels of iron and B12, both contribute to the production of collagen which has an essential role in the development of strong and healthy scar tissue (Fusano et al., 2020). Another study by the same researchers, investigated skin healing post photodynamic therapy for actinic kertosis and observed a hyperactive immune response in the habitual vegans (Fusano et al., 2021), see Table 2.1. Swelling and redness (both aspects of the inflammatory response) were more pronounced and prolonged compared to the omnivores, with a longer total skin healing time (Fusano et al., 2021). Similarly, the vegan participants in this study also had lower serum iron and B12 levels prior to treatment. It should also be noted that for both studies, the sample sizes were small and of an older population with possible confounding factors such as cardiovascular disease; therefore, the findings may not be applicable to young athletes.

It is plausible that there may be differences in the post-exercise inflammatory response between vegans and omnivores due to the high intake of some key nutrients and the evidence for lower systemic inflammation associated with plant-based diets. However, due to the current gaps in literature, further research on habitual dietary patterns is required.

Table 2.1: Characteristics of studies examining inflammation and wound healing in participants following plant-based or omnivorous dietary patterns.

Reference	Study Design	Population	Protocol	Outcome
(Craddock et al., 2019)	Systematic review and meta-analysis of 30 observational and 10 intervention studies	Vegetarian (vegan and vegetarians) and omnivores. Diet duration: >1 year. Majority healthy (2 studies with dialysis participants, 1 with CVD participants) Female & male Young to adult	Analysis of inflammatory biomarkers in habitual dietary patterns.	Pooled effects of vegetarian-based dietary patterns were associated with significantly lower concentrations of CRP, fibrinogen, and total leukocyte compared with those following non-vegetarian dietary patterns in observational studies. Insufficient data were identified for a meta-analysis of intervention studies.
(Craddock et al., 2023)	Cross-sectional	12 vegans 8 omnivores Diet duration: >3 years Healthy, endurance trained >4 hr /week training BMI range: 22-25 Males only Average age: 34	Increment ramp running at 60% and 90% VO2 peak. Analysis of inflammatory biomarkers. No dietary intervention.	No differences in CRP, fibrinogen, IgA, IL-2, IL-6, IL-10, IFN-γ, TNF-α were observed before or after running. Total red blood cell count, haemoglobin and haematocrit levels were lower in the vegan dietary group. No differences in white blood cells or platelets were observed.
(Eichelmann et al., 2016)	Systematic review of 29 studies	2,689 obese participants with medical conditions Female & male Average BMI: 29	Prescribed diets: mediterranean (*n*= 17), Nordic (*n*= 4), DASH (*n*= 2), vegetarian (*n*= 1), palaeolithic (*n*= 1) Duration: 5 to 96 weeks	Consumption of plant-based diets was associated with overall improvement in inflammatory profiles compared with control diets. The most pronounced effects were CRP, IL-6 and sICAM.
(Franco-de-Moraes et al., 2017)	Cross-sectional	268 participants following vegan, lacto-ovo-vege or omnivorous diets Healthy, female & male, average BMI: <30, average age: 49	Evaluated inflammatory status (CRP, LPS, IL-10, TNF-α, insulin resistance, and CVD risk.	Excess weight, pre-diabetes and hypotension were higher in omnivores. Inflammatory markers exhibited a gradual and significant increase from the vegans to lacto-ovovegetarians to omnivorous group.
(Fusano et al., 2020)	Prospective observational	21 vegans 21 omnivores Generally healthy (comorbidities excluded). Female & male	Recovery and healing observed for 6 months post < 2-cm diameter, nonmelanoma skin cancer excision	Vegans showed a significantly lower mean serum iron level (P= <.001) and vitamin B12 (P= <.001). Wound diastasis was more frequent in vegans (P= .008). After 6 months, vegan patients had a higher modified SCAR score than omnivores (P=

Reference	Study Design	Population	Protocol	Outcome
				<.001), showing the worst scar spread (P= <.001), more frequent atrophic scars (P= <.001), and worse overall impression (P= <.001).
(Fusano et al., 2021)	Prospective observational	30 vegans 30 omnivores Healthy Female & male Average age: 68	Observe skin healing and side effects post photodynamic therapy for actinic keratosis in habitual dietary patterns.	Vegans had a poorer recovery with a stronger and more prolonged inflammatory response (oedema, erythema and vesiculation) (P= <.001) and a significantly longer total wound healing time (P= <.001).
(Haddad et al., 1999)	Cross-sectional	25 vegans 20 omnivores Healthy. Female & male. BMI range: 18-29. Average age: 35	Evaluated nutritional and immune status.	Vegans had significantly lower leukocyte, lymphocyte, and platelet counts. Vegans did not differ from omnivores in functional immunocompetence assessed as mitogen stimulation or natural killer cell cytotoxic activity.
(Jenko Pražnikar et al., 2023)	Cross-sectional	24 vegans 21 vegetarians 24 omnivores 20 low CHO, high fat Diet duration: >6 months Healthy, female & male, average BMI: 22, average age: 36	Evaluated inflammatory status with IL-6, TNF-α and CRP measures.	No differences found in any of the parameters between the four groups and no strong correlations with dietary intakes.
(Menzel, Biemann, et al., 2020)	Cross-sectional	36 vegans 36 omnivores Diet duration: >4 years Healthy, female & male, BMI: < 30, average age: 38	Evaluation of inflammatory biomarkers in habitual diets.	No significant differences in CRP, IL-18, IL-1 RA, ICAM-1, adiponectin, omentin-1 and resistin. However, the duration of following a vegan diet (>4.8 years) was positively correlated with lower resistin, IL-18 and IL-1 RA concentrations.
(Richter et al., 1991)	Cross-over	8 habitual omnivores Healthy, endurance trained males only Age range: 21-28	6 weeks omnivore diet. 4 week wash out. 6 weeks lacto-ovo vegetarian. Evaluated aerobic capacity and immune cell activity.	No difference in maximal aerobic capacity and no differences in blood mononuclear cells or NK cell activity.

2.5 Summary

The acute inflammatory process following exercise-induced muscle damage (EIMD) is crucial for muscle repair and recovery. EIMD causes myofibre damage, triggering an immune response and inflammation brings fluid and immune cells to the injury site. Immune cells release cytokines that attract more immune cells, which phagocytose debris and release reactive oxygen species, promoting further degradation. Muscle regeneration is then facilitated by satellite cells, which are activated by EIMD and proliferate during the pro-inflammatory phase. These cells differentiate during the anti-inflammatory phase to repair and form new muscle fibres, restoring strength and function. However, balancing inflammation is critical, as excessive inflammation can lead to maladaptive recovery while suppressing it can hinder muscle regeneration. Recovery strategies like cold water immersion or adequate sleep may help manage inflammation, though other interventions show varying effectiveness.

Diet can significantly attenuate exercise-induced inflammation, with nutrients like proteins, carbohydrates, fats, and antioxidants playing crucial roles. Many studies on the dietary impacts on exercise and muscle recovery focus on nutrient supplementation, which often leads to varying results compared to nutrients derived from whole food sources. Although a vegan diet is typically higher in anti-oxidative nutrients, this benefit may be offset by the high fibre content, as fibre can lower polyphenol bioavailability, potentially leading to increased oxidative stress markers. Proteins, especially from animal sources, provide essential amino acids necessary for muscle regeneration, while plant-based proteins may lack some of these amino acids. Dietary fats have a dual role; saturated fats can worsen inflammation, whereas unsaturated fats have anti-inflammatory properties. Antioxidants, including vitamins and minerals such as zinc, help protect against muscle damage and support repair.

While the inflammatory response in omnivores and vegetarians post-exercise is well-studied, research on vegans is limited. Vegetarians tend to have lower systemic inflammation levels than omnivores, and it is plausible that vegans, with their lower saturated fat intake, may also experience reduced inflammation. However, it is unclear if this lower inflammation optimises the muscle repair process and enhances recovery, as a balanced pro-inflammatory phase is essential for clearing damaged cells but can cause additional tissue damage if prolonged. More research is needed to determine if vegans benefit from faster muscle recovery due to their potentially lower systemic inflammation levels, and whether nutrients from whole food sources can confer muscle recovery beneficial effects.

This study aims to determine if the source of macronutrients impacts the systemic inflammatory response or muscle damage markers post-exercise among healthy adults in Auckland, New Zealand. This study has three main objectives: control the participants' macronutrient intake based on the analysis of their habitual diet for 72 hours (energy at individual requirements, 1.4 - 1.6g/kg protein, 55% of energy in carbohydrates, remainder energy in fat); induce muscle fibre damage using an established drop jump exercise protocol; and compare the inflammatory response between participants by measuring plasma cytokine (IL-6, IL-10, and IL-1β) concentrations at hours 0, 1, 3, 24, 48, and 72 post-exercise protocol. Additionally, the study will compare muscle damage between participants by measuring plasma CKM levels at the same time points. Two highlights of this study are controlling for calories and macronutrient intake during the trial, a factor often overlooked in other studies, and basing the dietary control off the participants' habitual diets. The main dietary

difference between groups will be the protein source, comparing plant-based versus animal-based proteins. This approach aims to provide clearer insights into how different macronutrients from whole food sources affect inflammation and muscle recovery post-exercise.

Chapter 3 Manuscript

3.1 Abstract

Introduction: Exercise-induced muscle damage (EIMD) triggers an inflammatory response essential for muscle repair and recovery. The impact of habitual dietary patterns on this response is not well understood.

Aim: This study investigated whether nutrient source (i.e., vegan or omnivorous diet) has an impact on the systemic inflammatory response or muscle damage markers following an eccentric exercise protocol.

Methods: Three vegans (32.3 ± 5.78 years; 63.4 ± 16.9 kg; 173.7 ± 9.5 cm; 20.8 ± 3.4 BMI) and seven omnivores (25.0 ± 5.34 years; 70.9 ± 8.5 kg; 167.9 ± 8.2 cm, 24.1 ± 2.5 BMI) underwent an eccentric exercise protocol involving 200 drop jumps. Muscle damage marker CKM, and inflammatory biomarkers IL-1β, IL-6 and IL-10 were analysed from plasma collected at baseline and 0 h, 1 h, 3 h, 24 h, 48 h, 72 h post-exercise. Participants followed a macronutrient-matched meal plan and consumed a standardised meal post-exercise.

Results: IL-6 increased over time (P= 0.005), however there was no difference between diets (P= 0.225). IL-1β, IL-10 and CKM did not change over time (P= 0.371, P=0.097 and P=0.491, respectively) or differ between diets (P= 0.925, P= 0.821 and P= 0.296, respectively). Dietary analysis of participants' habitual diets showed vegans had significantly lower intakes of cholesterol (P= 0.007), and dietary folate equivalents (P= 0.040). Dietary analysis of participants' intake during the trial showed vegans had significantly lower intakes of cholesterol (P= 0.010), niacin equivalent (P= 0.028), and vitamin B6 (P= 0.032). Daily intake of fibre, folate, vitamin A, omega-3 polyunsaturated fatty acids, selenium, calcium, and zinc were below recommended levels for the vegans. Whereas daily intake of calcium was below recommended levels for the omnivores.

Conclusion: The study found no significant differences in systemic inflammatory or muscle damage markers between habitual vegan or omnivorous diets following eccentric exercise. Despite significant differences in nutrient intake between diets, the eccentric exercise protocol was not strenuous enough to produce notable systemic stress detectable by the biomarkers measured. The small sample size limited the study's power, highlighting the need for further research with larger samples and more comprehensive assessments, including, for example, muscle ultrasound for assessment of muscle damage, additional inflammatory biomarkers (e.g., IL-4 and IL-1ra), and plasma lipid panels and plasma nutrient levels. Future studies should continue to explore the effects of whole dietary patterns on muscle recovery and inflammation without inducing nutrient deficiencies or excessive supplementation.

Keywords: Exercise-induced muscle damage (EIMD), inflammatory response, habitual diet, vegan, omnivore, eccentric exercise, IL-6, CKM, IL-10, IL-1β.

3.2 Introduction

In recent years, veganism has seen a significant rise in popularity. One of the characteristics of veganism is a diet that excludes all animal products and emphasises plant-based foods such as vegetables, fruits, grains, and legumes (Eichelmann et al., 2016). This shift is driven by a combination of health considerations, environmental concerns, and ethical reasons related to animal welfare (Franco-de-Moraes et al., 2017). Despite its growing popularity, there is limited research on how a vegan diet influences inflammation and recovery from exercise-induced muscle damage (EIMD) (Owens et al., 2019). There is significant research into nutrient supplementation, however, understanding the effects of dietary patterns on inflammation and muscle recovery is mostly limited to omnivorous diets. Omnivore diets are predominantly animal product-based, with significantly different nutrient intakes compared to vegan diets. As more athletes choose to become vegan, it is crucial to understand the impact of this diet on the acute inflammatory response following EIMD, muscle tissue repair and the restoration of performance (Yang & Hu, 2018). Therefore, the aim of this study will be to assess whether inflammatory and muscle damage markers differ post-exercise between habitual vegans and habitual omnivores.

Exercise-induced muscle damage is typically caused by high-intensity or unfamiliar exercise, and it is associated with damaged muscle fibres, inflammation, and loss of performance. Cell content leakage from damaged fibres triggers the inflammatory response, attracting neutrophils and macrophages, which phagocytose damaged cells and release reactive oxygen species (ROS), thus creating secondary muscle damage (Owens et al., 2019). Muscle damage can be measured directly through histological analysis, ultrasound, MRI, and electron microscopy, or indirectly via markers like force production and plasma creatine kinase (CK) levels. In order to induce muscle damage in this study, a drop-jump exercise protocol was implemented, and damage was measured through plasma CKM levels at pre-exercise and 0 h, 1 h, 3 h, 24 h, 48 h, and 72 h post-exercise.

The inflammatory response following EIMD is essential for muscle repair and adaptations. Initially, during the pro-inflammatory phase, immune cells are attracted to the site of injury to phagocytose damaged fibres and activate muscle satellite cells (Fatouros & Jamurtas, 2016; Owens et al., 2019). Muscle regeneration occurs during the anti-inflammatory phase and involves the differentiation of satellite cells to repair fibres and restore muscle function (Chazaud, 2016; Peake et al., 2016). Throughout the pro- and anti-inflammatory process, immune cells express cytokines that act as intercellular messengers, regulate immune function and influence various other cell processes (Pillon et al., 2013).

Cytokines are crucial for muscle repair following exercise-induced damage and play pro- and anti-inflammatory roles in the recovery of function and muscle adaptions (Paulsen et al., 2012). The pro-inflammatory cytokine Interleukin-1β (IL-1β) recruits additional immune cells to the injury site, and has been shown to stimulate the production of interleukin-6 (IL-6) in skeletal muscle cells, suggesting a direct impact on muscle tissue (Yang & Hu, 2018). The pro-inflammatory actions of IL-6 include the recruitment of neutrophils and stimulation of muscle cells to release chemical signaling molecules that attract peripheral monocytes and T cells (Scheller et al., 2011). Il-6 levels decrease after the resolution of the pro-inflammatory phase; however, they remain elevated above baseline for a few days after. IL-6 triggers T cells to release the anti-inflammatory cytokine IL-10 and inhibits macrophage production of the pro-inflammatory cytokines TNF-α and IL-1β (Fatouros & Jamurtas, 2016; Kistner et al., 2022). IL-6 is involved in the activation, proliferation, and differentiation of

muscle satellite cells and, therefore, the formation of myotubes (Fatouros & Jamurtas, 2016; Pillon et al., 2013; Yang & Hu, 2018). IL-10 is an anti-inflammatory cytokine involved in immune regulation and tissue regeneration. IL-10 is initially secreted by T cells (Tregs), alongside other cytokines, to facilitate the conversion of M1 to M2 macrophages (Yang & Hu, 2018). IL-10 also suppresses the pro-inflammatory state by inhibiting cytokine IL-1β (Peake et al., 2016). To assess and compare the inflammatory response time course between vegan and omnivores, plasma concentrations of cytokines IL-1β, IL-6 and IL-10 was measured pre-exercise and 0 h, 1 h, 3 h, 24 h, 48 h, and 72 h post-exercise.

Various nutrients, including proteins, carbohydrates, fats and antioxidants, play indispensable roles in muscle repair and inflammation modulation. Skeletal muscle is regulated by muscle protein synthesis and breakdown, which is influenced significantly by dietary protein intake (Kerksick et al., 2021). Dietary protein provides the essential amino acids (EAAs) crucial for muscle protein synthesis, therefore, EAAs are vital for muscle regeneration, growth and overall function (Arentson-Lantz & Kilroe, 2021). Carbohydrates are the primary energy source during high-intensity exercise, delaying fatigue and improving performance by maintaining muscle and liver glycogen stores (Ivy, 2004). Post-exercise carbohydrate intake is needed to replenish glycogen stores, promote recovery and reduce muscle damage (Roy et al., 1997). Dietary fats exhibit a dual relationship, saturated fats can exacerbate inflammation, whereas unsaturated fats demonstrate anti-inflammatory properties (Franco-de-Moraes et al., 2017). Antioxidants encompass phytochemicals, vitamins and minerals and serve a crucial role in mitigating inflammation and oxidative stress (Bell et al., 2015). They can act as defence mechanisms against free radicals and other ROS generated from EIMD (Bell et al., 2015). Excessive ROS can exacerbate muscle injuries by damaging cells; antioxidants might mitigate these effects by neutralising these reactive molecules (Peake, Nosaka, et al., 2005).

These various nutrients have a significant effect on EIMD, inflammation, and muscle recovery. However, it is important to note that many studies are on the supplementation of these specific nutrients, and not on whole foods containing these nutrients or on entire dietary patterns such as veganism. Often, other food components can affect nutrient bioavailability and absorption (Liu et al., 2024). Therefore, the effects of diet on EIMD and muscle recovery cannot be anticipated by examining individual nutrients alone. It is essential to consider the whole diet to determine if the source of nutrients also plays a role.

In order to determine if nutrient source has an impact on the systemic inflammatory response or muscle damage markers post-exercise, this study compared vegan and omnivore diets with matched macronutrient and caloric intake. Each diet provided energy at individual requirements, 1.4 - 1.6 g/kg protein, 55% of energy from carbohydrates, and the remainder from fats.

3.3 Methods

3.3.1 Participants and recruitment

In this non-randomised, controlled, cross-sectional study, participants were recruited in 2023 by advertising on social media, email newsletter, Stream (Massey University student website), word of mouth, and Massey University notice boards (Appendix 1). This study was open to all genders and participants were included if they had followed their vegan or omnivore diet for more than two years, were aged 18-40 years old, and were physically independent with sedentary or general physical

fitness (exercised 1-3 days a week). Participants were excluded if they had any chronic diseases or underlying medical conditions, were receiving hormone replacement therapy or anabolic steroids, were on hormonal birth control, and were involved in heavy strength training or other high-force activities, including near maximal or maximal eccentric force generation. Interested participants were provided information sheets (Appendix 2), and after completing a health-screening questionnaire (Appendix 3) provided written informed consent before study commencement.

Participants were stratified into either the vegan or omnivore group according to their diet for the previous 2 years. A total of 10 participants took part in the study (Figure 3.1), 6 females and 4 males (see Table 3.4 for characteristics). Participants were instructed to avoid exercise from 48 hours prior to the competition of the trial, and abstain from consuming alcohol, caffeine, supplements, and anti-inflammatory medications from 24 hours prior until completion of the trial.

Ethical approval was provided by the Massey University Human Ethics Committee: Ohu Matatika 1, application OM1 23/06.

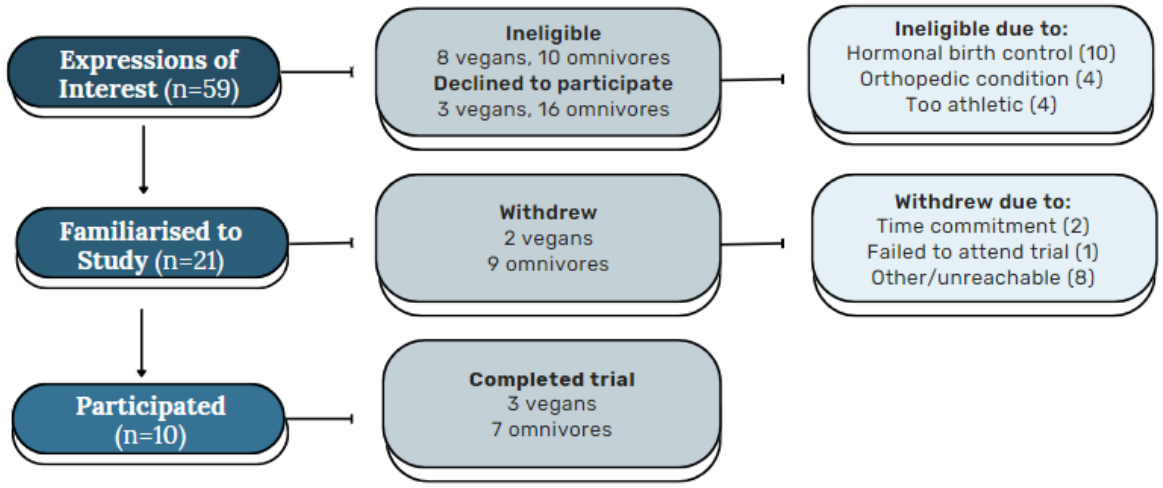

Figure 3.1: Flow diagram of recruitment

3.3.2 Experimental protocol

Participants were required to attend the laboratory for a familiarisation session before and for four consecutive days during the trial. The muscle-damaging protocol was undertaken on the first day, whereas the nutrition intervention and data collection were carried out for all four days of the trial. The total time involved was estimated to be between 9.75 – 10.25 hours (see Appendix 2 for the timings detailed).

Familiarisation session

Participants were required to attend a familiarisation session prior to commencing the trial to ensure informed consent was obtained. The protocol and data collection methods were explained, and participants completed one rep of the muscle damage protocol (1 drop-jump) and all of the data collection set activities (detailed below). The health screening questionnaire was completed (Appendix 3), and the consent form was signed (Appendix 4). Height and weight were recorded to calculate estimated dietary requirements.

Nutritional intervention

On the day of the muscle damaging protocol and the three subsequent data collection days, participants' diets were controlled to ensure macronutrient intake was comparable across the groups. All participants completed a 3-day food diary before the trial, which was analysed using Foodworks Professional (software package 2007, Xyris Pty Ltd, Australia) (Appendix 5). Participants were provided with a 4-day meal plan that resembled their typical intake (meal-type and frequency); however, serving sizes were altered to ensure comparable macronutrient intake and sufficient caloric intake. Macronutrient intake was prescribed as: energy intake at their predicted requirement (calculated with Cunningham Equation for resting metabolic rate), protein intake between 1.3-1.6 g/kg, carbohydrate 55% of total energy intake, and fat comprised the remainder of their energy intake. Participants were required to record a 4-day food diary during the trial to monitor adherence to the dietary protocol, and it was requested that any deviances be recorded.

On the day of the muscle-damaging protocol, participants arrived at various times from early to late morning according to their schedules. They were advised to follow their prescribed meal plans; and eat breakfast prior. All participants were provided a meal 1.5 hours after the completion of the exercise protocol. They were commercially made, frozen meals. The vegan meal was a Creamy "Chicken" Pasta (Harmless Food Co, New Zealand) and the omnivore meal was a Chicken Cordon Blue with potatoes, corn, green beans, and carrots (Wattie's, New Zealand). The macronutrients of the vegan and omnivore meals were similar (see Table 3.1), and the amount served to participants was controlled at 10 kcal/kg. Participants were free to drink water as desired; however, they were requested not to eat (other than the provided meal) until after the 3 h post-exercise data collection time point.

Table 3.1: Macronutrient content of controlled, post-exercise meal.

Macronutrients	Vegan Meal	Omnivore Meal
Energy	10 kcal/kg	10 kcal/kg
Protein (g)	26	23.1
Carbohydrate (g)	69	49.6
Fat (g)	15	26.9
Saturated Fat (g)	2	8

Muscle damage protocol

The muscle damage protocol consisted of 200 drop-jumps from a 0.6 m high box. Participants were encouraged to take 10 seconds to perform each jump, and a 2-minute rest break punctuated every 20 jumps. Once 100 jumps had been completed, participants rested for 5 minutes before completing the remaining 100 jumps in the same manner. The exercise protocol took on average 45 minutes to complete. Participants were instructed to step off from the box, land on two feet and immediately descend to a 90°-degree squat followed by a maximal effort vertical jump. A 100 drop-jump protocol has previously been shown to induce lower limb muscle damage (Howatson et al., 2012); however, in a similar 2022 study conducted by Massey University, no clear effect on muscle damage and inflammation was observed (Leach, 2023; Woolsey, 2023). Therefore, this study increased the total number of drop-jumps to 200.

3.3.3 Data collection

Data was collected at 7 time points: baseline (immediately prior to muscle-damaging exercise), 0 h (immediately post-exercise), and 1, 3, 24, 48, and 72 h post-exercise. The type of data collected at each timepoint was the same (see Figure 3.2), except for baseline, for which body composition (with a bioelectrical impedance analysis machine) and height (with a stadiometer) were also taken. This project was undertaken in collaboration with another Master of Science Nutrition and Dietetics student who focused on the recovery of muscle function. Pertinent data collected were counter-movement jump (CMJ), maximum voluntary isometric contraction (MVIC), subjective pain and pain pressure threshold (see Appendix 6 for data).

Figure 3.2: Type of data collected, in order of testing. CMJ: Counter movement jump. MVIC: Maximum voluntary isometric contraction.

Blood sampling and cytokine analysis

An 8.5 ml venous blood sample was obtained via venepuncture at each time point. Samples were collected in 10 ml vacutainer tubes treated with heparin as an anticoagulant. The samples were centrifuged at 4°C for 10 mins at 2,500 g immediately after collection. Triplicate plasma samples were aliquoted and stored at -80°C for later analysis. Inflammatory cytokines IL-1β, IL-6 and IL-10 were measured with ELISA kits: BMS224HS, BMS213HS, and BMS215-2HS, respectively (ThermoFisher Scientific, Waltham, MA, USA). Serum CKM was also measured by ELISA, according to the manufacturer's instructions (ab264617, Abcam, Cambridge, UK).

3.3.4 Statistical analysis

It was calculated that a minimum of 32 participants were needed for the study to reach a clinically significant difference at 80% power and 5% statistical significance using MVIC as the primary outcome based on variations shown in Clifford et al. (2016). Effect sizes (η^2) were calculated from the difference in group means divided by the standard deviation, with values of < 0.2 (negligible), 0.2 - 0.4 (small), 0.5 - 0.7 (medium), 0.8 - 1.2 (large), and > 1.2 (very large) (Sullivan & Feinn, 2012).

Statistical analysis was conducted using the IBM SPSS software version 29 (IBM corporation, NY, USA). Outliers were identified and excluded using Grubb's test (www.graphpad.com/quickcalcs/Grubbs1.cfm). Two-way Repeated Measures ANOVA test was used to analyse differences between vegans and omnivores in cytokine concentrations over time, as well as the time effect of the damaging exercise. Mauchly's Test of Sphericity was performed, and it was determined that the Greenhouse-Geisser correction was appropriate for all relevant data sets. Post Hoc Bonferroni test was conducted when significant differences were found by ANOVA. Independent 2-tailed t-test was used to evaluate differences between the vegan and omnivore groups in participant baseline characteristics and nutrient intake. The values were presented as the mean ± standard deviation. For all tests, a P-value <0.05 was considered statistically significant.

3.4 Results

3.4.1 Participants

Ten participants undertook the trial; however, one participant was excluded from data analysis as the blood draws for time points 0 h, 1 h, and 24 h could not be taken. No significant differences were found between the vegan and omnivore participants' baseline characteristics (see Table 3.2).

Table 3.2: Participant Baseline Characteristics

	Omnivore (n=6)	Vegan (n=3)	P-value*
Age (years)	25.6 ± 5.1	32.3 ± 5.8	0.135
Height (cm)	169.7 ± 7.2	173.7 ± 9.5	0.500
Weight (kg)	70.9 ± 8.5	63.4 ± 16.9	0.488
BMI (kg/m^2)	24.1 ± 2.5	20.8 ± 3.4	0.140
Muscle Mass (kg)	28.9 ± 4.4	28.4 ± 7.2	0.899
Fat Free Mass (kg)	52.3 ± 7.7	51.2 ± 11.8	0.868
Plasma CKM (ng/ml)	15.4 ± 2.7	15.2 ± 11.6	0.980

Values are mean ± SD.
*Significant differences between the two diet groups (P= <0.05), (Independent 2-tailed T-test).

3.4.2 IL-1β

Plasma IL-1β concentrations did not change significantly over time, with a small effect size, in response to the muscle-damaging protocol ($F_{5,35}$= 1.071, P= 0.371, η^2= 0.133), see Figure 3.3. No significant difference for diet was found, with a small effect size ($F_{1,7}$= 0.010, P= 0.925, η^2= 0.001).

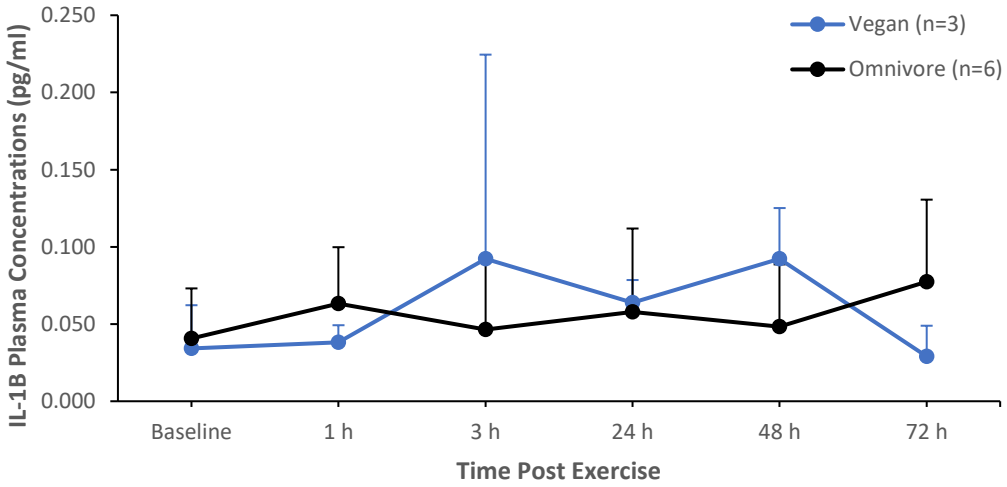

Figure 3.3: Plasma IL-1β concentrations (pg/ml) over time after muscle damaging protocol. Data was analysed with Two-way Repeated Measures ANOVA. Values presented as mean ± SD. Time effect: $F_{5,35}$= 1.071, P= 0.371, η^2= 0.133. Diet type effect: $F_{1,7}$= 0.010, P= 0.925, η^2= 0.001. Diet and time interaction: $F_{5,35}$= 2.356, P= 0.129, η^2= 0.252.

3.4.3 IL-6

Plasma IL-6 concentrations did change significantly over time, with a medium effect size, in response to the muscle-damaging protocol ($F_{5,30}$= 7.414, P= 0.005, η^2= 0.553), although no specific differences from baseline were detected using Post Hoc Bonferroni test. Plasma levels peaked at 3 h for both groups, with a larger increase seen in the vegan participants, before returning to baseline levels at 72 h, despite that no significant difference for diet was found, with a small effect size ($F_{1,6}$= 1.826, P= 0.225, η^2= 0.233), see Figure 3.4.

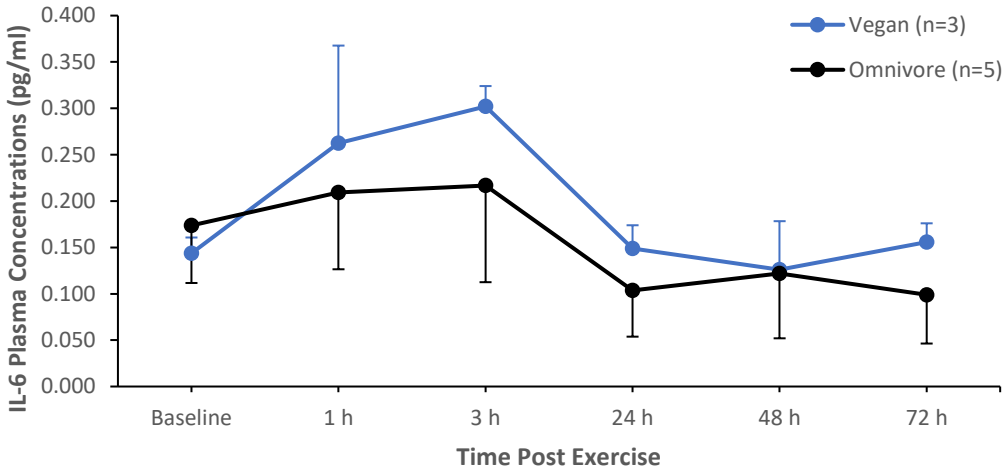

Figure 3.4: Plasma IL-6 concentrations (pg/ml) over time after muscle damaging protocol. Data was analysed with Two-way Repeated Measures ANOVA. Values presented as mean ± SD. Time effect: $F_{5,30}$= 7.414, P= 0.005, η^2= 0.553. Diet type effect: $F_{1,6}$= 1.826, P= 0.225, η^2= 0.233. Diet and time interaction: $F_{5,30}$= 0.862, P= 0.458, η^2= 0.126. Results from one omnivore participant was excluded as an outlier.

3.4.4 IL-10

Plasma IL-10 concentrations did not change significantly over time, with a small effect size, in response to the muscle-damaging protocol ($F_{5,35}$= 2.503, P= 0.097, η^2= 0.263), see Figure 3.5. No significant difference for diet was found, with a small effect size ($F_{1,7}$= 0.055, P= 0.821, η^2= 0.008).

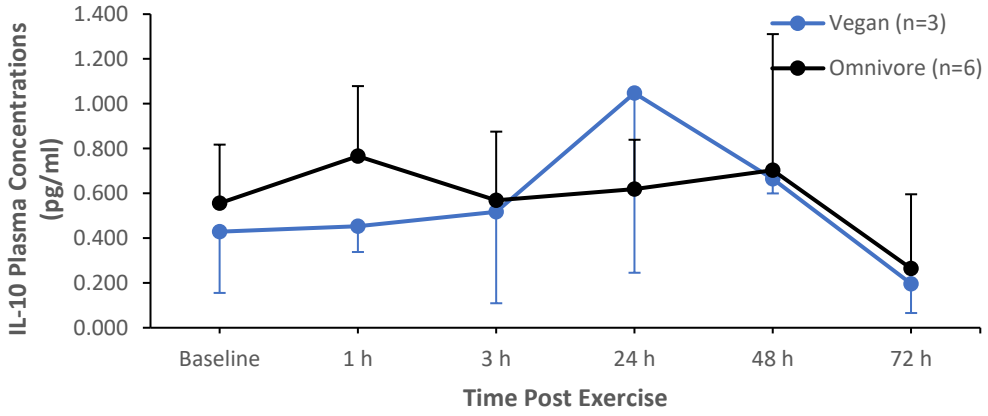

Figure 3.5: Plasma IL-10 concentrations (pg/ml) over time after muscle damaging protocol. Data was analysed with Two-way Repeated Measures ANOVA. Values presented as mean ± SD. Time effect: $F_{5,35}$= 2.503, P= 0.097, η^2= 0.263. Diet type effect: $F_{1,7}$= 0.055, P= 0.821, η^2= 0.008. Diet and time interaction: $F_{5,35}$= 0.918, P= 0.441, η^2= 0.116.

3.4.5 Creatine kinase

Plasma CKM concentrations did not change significantly over time, with a small effect size, in response to the muscle-damaging protocol ($F_{5,25}$= 0.739, P= 0.491, η^2= 0.129), see Figure 3.6. However, there is a noticeable increase in CKM levels for the vegan group at the 1 h timepoint, from which it plateaus and returns close to baseline values at 48 h. Whereas, the omnivorous group have a slight rise at 24 h before returning to baseline at 72 h. Despite an apparent higher total value in the vegan group, there was no significant difference for diet found, with a small effect size ($F_{1,5}$= 1.360, P= 0.296, η^2= 0.214).

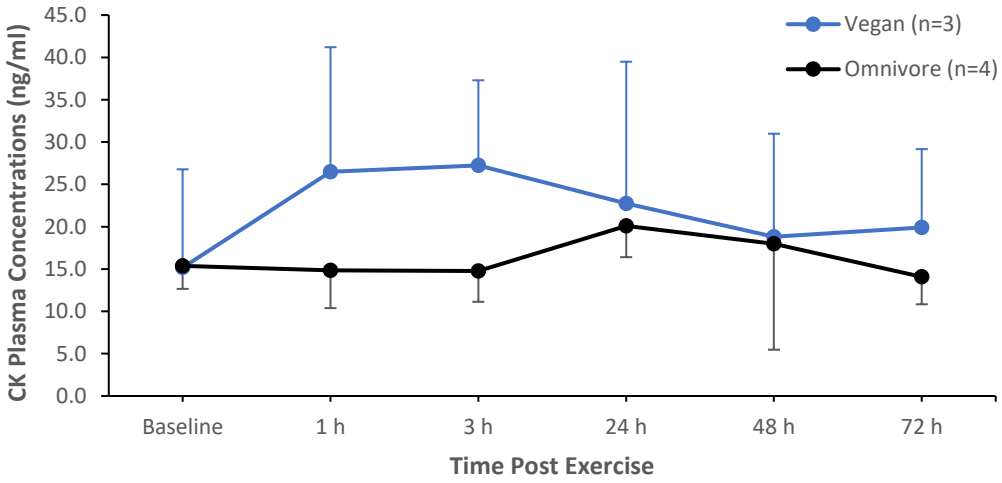

Figure 3.6: Plasma CKM concentrations (pg/ml) over time after muscle damaging protocol. Data was analysed with Two-way Repeated Measures ANOVA. Values presented as mean ± SD. Time effect: $F_{5,25}$= 0.739, P= 0.491, η^2= 0.129. Diet type effect: $F_{1,5}$= 1.360, P= 0.296, η^2= 0.214. Diet and time interaction: $F_{5,25}$= 0.888, P= 0.434, η^2= 0.151. Results from two omnivore participants were excluded due to missing timepoints.

3.5 Discussion

This study explored the inflammatory response post-eccentric leg exercise in healthy adults consuming a habitual vegan or omnivorous dietary pattern with matched macronutrient and caloric intake. This study found that despite differences in nutrient sources (i.e., vegan vs. omnivores) and micronutrient intake, there were no significant differences between dietary groups either at baseline or after the exercise protocol. Muscle mass and fat free mass were similar for the groups. There were no significant differences in IL-10, IL-6, IL-1β, and plasma CKM between the groups either pre- or post-exercise. However, there was a significant increase in IL-6 concentrations over time post-exercise in both groups.

3.5.1 Inflammatory response to the exercise protocol

This study found no significant increase in plasma CKM levels post-exercise, which suggests the protocol was insufficient at inducing significant muscle damage. Although both groups experienced an increase in plasma IL-6 levels over time, IL-10 and IL-1β remained low and did not change after exercise. This indicates that the exercise protocol was ineffective in inducing a large systemic inflammatory response, aligning with the low levels of plasma markers of muscle damage observed.

These findings differ from previous studies that have utilised the drop-jump protocol, where increases in plasma CKM and IL-6 levels were observed. Creatine kinase is an enzyme primary found

in tissues with high energy demands, such as muscle (de Castro et al., 2011). Muscle cell membrane damage allows CK to leak into the circulation, causing plasma CK levels to rise and peak approximately 24 to 72 hours post-exercise (Brancaccio et al., 2006). For instance, in the beetroot juice supplementation study, 30 recreationally active males performed 100 drop-jumps with EIMD parameters measured at pre-exercise and 24, 48 and 72 hours post-exercise (Clifford et al., 2016). Plasma CK increased, peaking at 24 hours, while IL-6 also elevated immediately post-exercise, returning to baseline by 24 hours. In another study, 24 healthy, recreationally active males performed a plyometric protocol consisting of 50 jumps over 50 cm hurdles and 50 drop-jumps (Chatzinikolaou et al., 2010). Plasma CK significantly increased at 24 hours, peaking at 48 hours and returning to baseline at 72 hours. IL-6 concentration also increased twofold immediately post-exercise, peaking at 24 hours and returning to baseline at 48 hours (Chatzinikolaou et al., 2010). In contrast, this current study found no significant difference in plasma CK levels over time, but it is important to note that the sample size was underpowered at only 10 participants. As discussed in section *3.3.2 Participants and Recruitment*, 32 participants were needed for this study to reach a clinically significant difference at 80% power and 5% statistical significance. Due to this small sample size, this project could be considered as a pilot study.

Despite a significant rise in IL-6 levels, the key inflammatory cytokines IL-1β and IL-10 were not significantly altered over time, suggesting the exercise-induced stress was insufficient to generate a measurable inflammatory response. The pro-inflammatory cytokine IL-1β is mainly produced by macrophages, it recruits immune cells to injury sites and stimulates IL-6 production in skeletal muscle cells (Lopez-Castejon & Brough, 2011; Yang & Hu, 2018). While IL-1β is evident in systemic inflammation post-EIMD and remains elevated into the anti-inflammatory phase, potentially aiding muscle regeneration by activating satellite cells (Fatouros & Jamurtas, 2016), the lack of change in this study suggests a minimal inflammatory response.

Similarly, IL-10, an anti-inflammatory cytokine crucial for immune regulation and tissue regeneration, did not significantly change. IL-10 local expression typically increases post-exercise, though plasma concentrations vary among studies and individuals (Paulsen et al., 2012). IL-10 is initially secreted by T cells and aids in converting M1 macrophages to M2 macrophages (Steen et al., 2019; Yang & Hu, 2018). This shift promotes myoblast proliferation and differentiation, facilitating muscle regeneration and growth (Pillon et al., 2013). IL-10 also suppresses inflammation by deactivating M1 macrophages and inhibiting IL-1β, which triggers IL-6 secretion and inhibits myogenin (Peake et al., 2016).

IL-6 plays a dual role in exercise-induced responses, exhibiting both pro- and anti-inflammatory actions that are pivotal in the immune response and muscle adaptation. The release of IL-6 during exercise is influenced by several factors. Greater increases in IL-6 levels are seen with high intensity and longer duration exercises, as well as energy availability such as low muscle glycogen levels (Craddock et al., 2020; Pedersen & Febbraio, 2008). IL-6 initially facilitates the clearance of damaged tissue by attracting leukocytes and enhancing phagocytosis (Scheller et al., 2011). As the inflammatory response progresses, IL-6 shifts to an anti-inflammatory role by aiding the clearance of neutrophils and triggering T cells to release IL-10, which in turn inhibits the production of TNF-α and IL-1β (Kistner et al., 2022).

Importantly, IL-6 release is not limited to tissue damage. Non-damaging triggers such as muscle contraction and low muscle glycogen significantly contribute to IL-6 secretion (Pedersen et al., 2004;

Pedersen et al., 2001; Pillon et al., 2013). Muscle contraction increases calcium influx, activating the calcineurin/NFAT pathway and enhancing IL-6 gene expression (Febbraio & Pedersen, 2002). Additionally, calcium and reactive oxygen species (ROS) activate the NF-κB pathway, further promoting IL-6 production (Fischer, 2002). During intense exercise, lactate production activates proteases that release IL-6 stored within muscle fibres (Hojman et al., 2019). Beyond its inflammatory functions, IL-6 regulates energy metabolism by enhancing lipolysis, increasing glucose uptake and stimulating gluconeogenesis (Pedersen & Febbraio, 2008; Pillon et al., 2013). IL-6 is also crucial for muscle adaptation to exercise, promoting satellite cell activation, muscle repair, growth, and supporting muscle protein synthesis while reducing muscle protein breakdown (Fatouros & Jamurtas, 2016; Pedersen & Febbraio, 2008).

In a study with 5 moderately active males who performed eccentric exercises, plasma IL-6 concentrations did not peak simultaneously with markers of muscle damage (serum myoglobin and DOMS symptoms) (Croisier et al., 1999). These findings suggest that the post-exercise increases in IL-6 may be driven more by metabolic factors than by inflammation, aligning with the non-damaging mechanisms described above and corroborating the lack of muscle damage observed in this current study.

Other studies on inflammation post-exercise with eccentric movements that were not drop jumps reported varying changes in systemic levels of plasma IL-10 and IL-1β, although IL-6 levels typically increased. In a high-intensity eccentric exercise study, 6 healthy, untrained young males performed bench presses and leg curls at 100% maximum effort (Smith et al., 2000). Plasma IL-6 increased significantly at 12, 24 and 72 hours post-exercise, while plasma IL-10 also significantly increased at 72 and 144 hours. However, plasma IL-1β was significantly decreased at 6, 24, and 144 hours post-exercise. In contrast, Fraschetti et al. (2022) observed no significant changes over time for IL-10, IL-6 and IL-1β after two bouts of high-intensity combined resistance and plyometric exercises with 13 healthy young females. Similarly, in a study with 13 recreationally resistance-trained women who performed 300 single leg extensions, Levitt et al. (2017) found no change in plasma IL-6 and IL-1β but observed a significant decrease in IL-10, despite a significant increase in plasma CK over time. In an eccentric elbow flexor exercise study with 10 healthy, young untrained male participants, IL-10 significantly increased while plasma IL-1β did not change over time (Hirose et al., 2004). Finally, Peake, Nosaka, et al. (2005) observed a significant increase in plasma IL-10 concentrations after high-intensity level treadmill running at 85% VO2 max for 1 hour with 9 well-trained male runners.

The lack of significant changes in plasma IL-1β in this and other studies suggests it may not be an appropriate biomarker for assessing local inflammation. Despite being a key inflammatory cytokine, it appears that circulating concentrations of IL-1β often do not increase significantly after EIMD (Peake et al., 2015). In three of the studies mentioned above plasma IL-1β did not significantly change post-exercise (Fraschetti et al., 2022; Hirose et al., 2004; Levitt et al., 2017). Therefore, local IL-1β concentrations might be better assess through muscle biopsies at the site of injury.

The variability in IL-10 responses appears to be attributable to exercise type. A systematic review assessing the response of IL-10 after acute exercise in healthy adults found that different types of exercise elicit varying IL-10 levels, with high intensity intermittent and aerobic exercises stimulating a greater increase in plasma IL-10 than strength (eccentric) exercises (Cabral-Santos et al., 2019). Therefore, the lack of significant difference in plasma IL-10 concentrations in this current study may

be attributed to the eccentric exercise protocol, indicating that IL-10 might not be an ideal biomarker for this context.

The co-researcher to this current study, Duncan (2024), used the same cohort and protocol to assess muscle function recovery post-exercise. Significant decreases in force (MIVC: P= 0.026), performance (jump height: P= 0.002), and increases in perceived pain (P= < 0.001) were observed for both vegan and omnivore groups (see Appendix 7). These findings indicate that the protocol effectively induced muscle function impairment and initiated a recovery process. The observed drop in force and increase in soreness were not clearly linked to inflammation or damage, suggesting that pronounced inflammation or extensive muscle damage is not necessary to impair muscle function post-exercise.

This finding is supported by other studies. For example, Yu et al. (2002) conducted various eccentric exercises (downstairs and downhill running, and eccentric cycling) with 35 healthy young adults. Muscle biopsies were taken to measure fibre damage, and despite participants reporting severe DOMS post-exercise, no damage was evident after eccentric contractions. In another study on the relationship between DOMS and indicators of muscle damage with 110 healthy young males, participants performed 12, 24 or 60 maximal eccentric elbow flexions (Nosaka et al., 2002). Muscle force, range of motion, muscle soreness, plasma CK and limb swelling were measured up to 4 days post-exercise. The author found weak evidence that indirect markers of muscle damage correlated with DOMS. In a review of literature, Malm (2001) found no evidence of inflammation and necrosis in muscle following EIMD, as many of the studies did not withstand critical review. The main flaw in the study designs being cited as the lack of a non-exercising control group, as it was speculated that the necrosis and inflammation was likely attributable to the invasive sampling procedures.

3.5.2 Inflammatory response to dietary differences

The lack of differences in baseline inflammatory biomarkers between the two groups is in contrast to literature associating plant-based dietary patterns with lower systemic inflammation (Craddock et al., 2019). This could be explained by this study's underpowered sample size; however, it is also likely attributable to the diets of the participants (see Appendix 6). There were significant differences in the average intake of cholesterol, folate, niacin, and vitamin B6 between the groups. Also of note was the inadequate intake of certain nutrients compared to the Nutrient Reference Values for New Zealand and Australia. These guidelines are typically Recommended Daily Intake (RDI) or Adequate Intake (AI). RDI is "The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group."; whereas, AI is "The average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate." (Australian Government Department of Health and Aged Care & Ministry of Health). The vegan group had an average inadequate intake of fibre, folate, vitamin A, omega-3 polyunsaturated fatty acids, selenium, calcium, and zinc. Whereas the omnivore group had an average inadequate intake of calcium only.

There are various fatty acid classes, with differing effects on the human body. Saturated fat can induce a pro-inflammatory state (Franco-de-Moraes et al., 2017), and intake is typically reported to be lower in vegan diets (Menzel, Jabakhanji, et al., 2020); however, there was no significant difference in habitual saturated fat intake between the two groups, and total fat intake was also similar. Omega-3 polyunsaturated fatty acids (n-3 PUFA) affect the activation of transcription factors and the expression of genes associated with inflammatory pathways, lipid metabolism, and

adipogenesis; therefore, potentially reducing inflammation and modulating the immune response (Calder, 2015). There was no significant difference in habitual intake of n-3 PUFA between the two groups; however, the average intake for the vegan group was below the suggested Adequate Intake (AI) standard. Research indicates that n-3 PUFA deficiency is more critical in the developing life stages such as pregnancy and infancy and is characterised by delayed and impaired neurological development and vision (Cassidy et al., 2009). In adults, the impact of inadequate intake is less clear; however, studies have shown n-3 PUFA supplementation to have a positive effect on muscle function and inflammation post exercise by attenuating inflammatory cytokines, oxidative activity and muscle damage (Owens et al., 2019).

Cholesterol is classified into two main types, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Watson & Meester, 2016). Both types are important for normal physiological functions; however, a higher ratio of HDL is preferred as it clears excess cholesterol from the body, and an excess of LDL is associated with negative health outcomes such as cardiovascular disease (Watson & Meester, 2016). Elevated LDL, especially when oxidised (oxLDL), activates macrophages and stimulates the production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 (Håversen et al., 2009; Watson & Meester, 2016) and contributes to atherosclerosis. Research is lacking on the impact of dietary cholesterol on muscle recovery post-exercise. However, Riechman et al. (2009) emphasise the importance of cholesterol in the repair of skeletal muscle and even hypothesise that elevated cholesterol may contribute to greater hypertrophy through the facilitation of inflammation. The habitual dietary cholesterol intake of the omnivore group in this study was significantly higher than the vegan group, this finding aligns with literature which reports intake to be lower in vegan diets compared to omnivorous diets (Haddad et al., 1999). However, without a lipid panel to measure total cholesterol, LDL and HDL, it cannot be ascertained how these dietary intakes translate to the participants' cholesterol status.

Zinc is essential for the proper functioning of enzymes and the immune response, and it also provides protection against oxidative stress and inflammation by modulating key pathways and cellular processes (Hernández-Camacho et al., 2020). Daily zinc intake is necessary as the human body lacks a storage system for the ion; there are concerns that the vegan diet may not provide sufficient zinc as it is found in highest concentrations and bioavailability in animal products (Thompson, 2017). Zinc deficiency is associated with poor immunity and increased oxidative stress (Maywald et al., 2017), and in chronic deficiency the production of pro-inflammatory cytokines (IL-6, IL-8, TNF-α) increases (Bonaventura et al., 2015). The average zinc intake of both groups during the trial period was comparable at 11.9 mg; however, plant-sourced zinc has poorer bioavailability due to the high phytic acid content, an inhibitor of zinc absorption (Hunt, 2003). The groups' average intake is also below the RDI of 14 mg/d for men (National Health and Medical Research Council et al., 2006). However, without directly assessing plasma zinc levels, it is difficult to ascertain zinc status and whether this inadequate intake translates to deficiency.

Vitamins C and E are classed as antioxidants, theorised to be able to prevent cell damage by scavenging free radicals and several studies have investigated whether supplementation can offset EIMD (Owens et al., 2019). Findings are equivocal, with some studies suggesting benefits and others suggesting no benefit, to negative outcomes. Most research seems to indicate that the inhibition of ROS from large dose supplementation may impair performance and muscle adaptations (Owens et al., 2019; Urso, 2013). However, it should be emphasised that many studies are supplementing with

dosage amounts significantly higher than the typical dietary intake. Surprisingly, habitual vitamin C intake was much lower for the vegan group compared to the omnivores, almost reaching significance (P= 0.052); however, intake for both groups was above the RDI of 45 mg/d (National Health and Medical Research Council et al., 2006). Conversely, habitual vitamin E intake was similar between the groups and above the RDI of 10 mg/d for men (National Health and Medical Research Council et al., 2006).

Folate, also an antioxidant, is crucial for many bodily functions, of relevance are red blood cell formation, skeletal muscle cell development, and amino acid metabolism including homocysteine, high levels of which are associated with inflammation and oxidative stress (Thompson, 2017). There is limited research in the role of folate sufficiency in muscle recovery post-exercise; however, the relationship of folate and skeletal muscle has been investigated. A study with >65 year olds found folate deficiency to be significantly correlated with reduced grip and leg strength (Wee, 2016). A deficiency of folate has been shown to inhibit muscle cell proliferation and differentiation, reduce the expression of myogenic markers, and promote cellular senescence (Hwang et al., 2019). The average habitual intake of dietary folate was significantly less for the vegan group (P= 0.040), and below the RDI of 400 µg/d (National Health and Medical Research Council et al., 2006). This contrasts with literature which reports folate intake to be typically higher in vegan diets compared to omnivorous diets (Wegmüller et al., 2017).

Selenium is a trace element and is incorporated into functional proteins as the amino acid selenocystenine (Bendich et al., 2004). These proteins have many roles; however, in relevance to the immune system is the prevention of oxidative damage to DNA, proteins, and cell membranes by scavenging free radicals, and the modulation of eicosanoid (signalling molecule) pathways (Bendich et al., 2004). Selenium deficiency, or supplementation, in the context of muscle recovery post-exercise has not been extensively studied. A systematic review of 6 studies investigated selenium supplementation on antioxidant systems, muscle damage markers and athletic performance with a varied healthy population of young adults, from trained to recreationally active (Fernández-Lázaro et al., 2020). Dosage ranged from 180-240 µg once daily and was in the form of either organic selenomethionine or inorganic sodium selenite. The average study duration was 10 weeks, with endurance training performed 3 times per week. This review found that compared to placebo controls, selenium supplementation significantly decreased lipid hydroperoxide levels and increased glutathione peroxidase in plasma, erythrocyte and muscle. However, no significant differences were observed on performance, training-induced adaptations on oxidative enzymes, muscle damage markers, or on muscle fibre type myosin heavy chain expression (Fernández-Lázaro et al., 2020). A study conducted by Milias et al. (2006), investigated whether baseline serum selenium status (i.e., sufficient or deficient) affects the overall response to injury. Thirteen healthy, recreationally active male participants performed 36 maximal eccentric actions with the elbow flexors on a motorised dynamometer. No supplementation was provided and the participants' diets were not modified. Selenium dietary intake was estimated with a food frequency questionnaire. Despite all participants meeting their dietary selenium RDI, 11 participants had serum selenium levels below the recommended value (Milias et al., 2006). Baseline serum selenium status was found to be associated with markers of EIMD. Higher serum selenium levels were linked to less muscle damage (lower CK and lactate dehydrogenase) and better muscle function (higher strength and range of motion) (Milias et al., 2006). In this current vegan vs. omnivores study, habitual dietary intake of selenium did not differ significantly between the two groups (P= 0.148); however, the average vegan intake was 32 -

42% below the RDI (National Health and Medical Research Council et al., 2006). It cannot be said whether the vegan group were deficient in selenium without testing serum; however, it is possible that their CKM levels post-exercise were affected by this inadequate dietary intake.

During the trial period there was a significant difference in the average intake of vitamins B6 and B3, with the vegan cohort consuming less than the omnivores. Niacin (vitamin B3) demonstrates many anti-inflammatory effects such as counteracting oxidative stress, and pathway and gene expression modulation which could inhibit the production of pro-inflammatory cytokines TNF-α and IL-6 (Rad et al., 2024). In a systematic review of niacin's effect on cytokines, the pooled results found a significant reduction in TNF-α; however, no statistically significant reduction in IL-6 levels (Rad et al., 2024). Niacin inhibits lipolysis, reducing free fatty acids which have been known to promote a pro-inflammatory environment (Rad et al., 2024). There is emerging evidence that niacin can also improve skeletal muscle mass and strength, and in a study with participants >40 years old it was found that dietary niacin intake was significantly positively correlated with grip strength and lean mass (Xiang et al., 2023). Vitamin B6 acts as a cofactor for many enzymes involved in metabolism and immune function (Morris et al., 2010), and is necessary for the production and regulation of cytokine levels (Doke et al., 1998). Studies have found that higher intakes of vitamin B6 is linked to decreased inflammation (Morris et al., 2010) and an enhancement of lymphocyte proliferation (Kwak et al., 2002). In a study on osteoarthritis (OA) in mice, it was found that daily 4-week vitamin B6 administration blocked the progression of OA and levels of IL-6 and TNF-α were significantly reduced (Fang et al., 2024). These B vitamins exhibit a convincing influence on inflammation; however, it is unlikely that the significant difference in vitamin B6 and B3 intake during the trial had an effect on the participants' inflammatory response because there was no significant difference in habitual intake between the groups. In the average habitual and trial intake, the groups consumed above the RDI for both B vitamins.

Vitamin A is a fat-soluble vitamin that has important roles in immune function and cell growth; however, the effects on muscle recovery post-exercise are unclear. Vitamin A deficiency results in impaired neutrophil and natural killer cell function, and lower lymphocyte counts (Semba, 2004). Retinoids are derivatives of vitamin A and have been shown to significantly influence the number and activity of macrophages, with deficiency impairing their phagocytic function (Semba, 2004). In a study on skeletal muscle injury in mice, it was found that vitamin A injections at the site of injury resulted in significantly accelerated repair of skeletal muscle (Zhang et al., 2023). Whereas, in a different study on rats subjected to intense swimming, it was found that vitamin A supplementation did not modulate IL-1β and TNF-α levels, and in fact, attenuated the antioxidant effect of exercise by approximately 50% and causing oxidative damage to the skeletal muscle lipids and proteins (Petiz et al., 2017). It is possible that these findings could be attributed to over dosing, as previous studies have reported negative effects from high vitamin doses on the inflammatory response and muscle adaptation (Clifford et al., 2016; Peake et al., 2007; Urso, 2013). In this current study, there was no significant difference in average habitual intake of vitamin A equivalents between the vegan and omnivore groups. However, the vegan group consumed on average 494 µg vitamin A which is far below the RDI of 900 µg (National Health and Medical Research Council et al., 2006). While research on vitamin A and exercise recovery is lacking, vitamin A has an intrinsic role in the immune system; therefore, we can speculate that deficiency would have an impact on the inflammatory response post-exercise. It is important to note that inadequate nutrient intake does not necessarily translate

to nutrient deficiency, as the body has the capacity to store enough vitamin A to last for several months (Semba, 2004), and nutrient status can be measured via a plasma sample (Lukaski, 2004).

Due to dietary differences, it was anticipated that the omnivore group would have higher systemic inflammation and a more pronounced pro-inflammatory response post-exercise. According to literature, vegan diets rely on vegetables, fruit, grain, legumes and nuts; and are typically high in dietary fibre, carbohydrates, and antioxidants such as vitamins and phytochemicals (Haddad et al., 1999; Menzel, Jabakhanji, et al., 2020). However, they are at risk of inadequate intake of vitamin B12 and D, iron, calcium, zinc, n-3 PUFAs, and protein (Craig, 2009; Thompson, 2017). Whereas omnivore diets are predominantly animal-product based and typically provide these nutrients, and often higher saturated fat, but may lack folic acid, magnesium, vitamins C, E, and niacin (Appleby & Key, 2016; Craig, 2009; Wegmüller et al., 2017). Contrary to literature, the omnivores in this current study demonstrated a higher average intake of the anti-inflammatory nutrients n-3 PUFAs, zinc, selenium, folate, and vitamins A, B3, B6 and C (see Appendix 6). Importantly, the vegan group often did not meet their daily requirements for many immunity-crucial micronutrients, such as n-3 PUFAs, folate, vitamin A, zinc, and selenium. It is possible that these dietary discrepancies offset any favourable inflammatory effects. However, many studies on the effects of nutrients in muscle recovery post-exercise are conducted with additional supplementation or with groups on nutrient-deficient diets, thus research into the effects of whole dietary patterns is lacking. Therefore, it is also possible that without additional nutrient supplementation, there are no post-exercise inflammatory differences in healthy participants following vegan or omnivore dietary patterns.

3.6 Conclusion

This study found that the levels of inflammatory biomarkers in plasma were similar between the vegan and omnivorous groups pre- and post-exercise. Neither diet or exercise produced significant differences in plasma CKM, IL-10, IL-6, and IL-1β. However, there was a significant increase in IL-6 concentration over time post-exercise, without differences between groups.

The eccentric exercise protocol did not induce a pronounced systemic stress response that was measurable by blood inflammatory or muscle damage markers. Participants responded similarly to the exercise protocol, and as the co-researcher Duncan (2024) found, they showed equivalent reduction in force and an inducement of pain (see Appendix 7). Therefore, while the protocol did have an effect on performance, an extended exercise duration or increased intensity may be required to induce a detectable increase in systemic inflammatory markers, or measurement of local biomarkers may be more appropriate (e.g., muscle biopsy: histological changes, leucocyte infiltration, *in-situ* cytokine production). Additionally, this project had a strong limitation with a small sample size which meant the findings were underpowered, and may have introduced bias. It was calculated that a minimum of 16 participants per group were needed for the study to reach a clinically significant difference at 80% power and 5% statistical significance using MVIC as the primary outcome based on variations shown in Clifford et al. (2016). However, this study concluded with only 3 vegans and 7 omnivores having participated in the trial. Due to this small sample size, this project could be considered as a pilot study.

Several nutritional disparities were noted between the groups that could potentially influence systemic inflammation and recovery post-exercise. The vegan group exhibited lower intakes of

folate, vitamin A, n-3 PUFAs, selenium, and zinc, which are all associated with lower systemic inflammation. Conversely, the omnivore group had higher cholesterol intake which is associated with increased systemic inflammation (Håversen et al., 2009; Watson & Meester, 2016). These nutrients are involved in immune function, inflammation regulation and overall general good health. However, many studies on the effects of nutrients in muscle recovery post-exercise are conducted with supplementation or with groups on nutrient-deficient diets. Research into the effects of whole dietary patterns including nutrient interactions, competition and bioavailability, without inducing deficiencies or over supplementing with excessive doses, is essential to better understand whether athletes might benefit physiologically from following a vegan or omnivorous diet. Further research with larger sample sizes and more detailed assessments, such as lipid panels and plasma nutrient levels, is also necessary to fully understand the implications of these dietary patterns on inflammation and muscle recovery post-exercise. There was no clear effect of nutrient dietary source on muscle recovery from the protocol chosen for this study.

Chapter 4 Achievements and Recommendations

4.1 Achievement of Aims and Objectives

This study has achieved its aim to compare the effects of nutrient source (vegan vs. omnivore) on the inflammatory response and muscle damage post-eccentric exercise among healthy adults following a habitual vegan or omnivorous dietary pattern.

This project achieved its objectives to compare the inflammatory response and muscle damage between participants both pre- and post-exercise (0 h, 1 h, 3 h, 24 h, 48 h and 72 h), while controlling participant macronutrient and caloric intake for the duration of the study.

This study assessed key pro- and anti-inflammatory plasma cytokines and plasma muscle damage markers. No significant differences were found between groups in the baseline systemic inflammatory status, or in the post-exercise inflammatory response and muscle damage markers following a single bout of eccentric leg exercise in healthy, adult vegans and omnivores.

4.2 Strengths

A key strength of this study was controlling the nutrient intake of participants on the day of the trial, as well as the 72 hours following. Macronutrient and caloric intake of participants were matched between groups (relative to the lean mass and body weight) to ensure no participants were either advantaged or disadvantaged by discrepancies in energy, protein, or carbohydrate intake. This was crucial as the aim of this project was to determine if the nutrient source, not nutrient amount, influenced muscle recovery and inflammation caused by EIMD. The controlled diets were designed from participants' habitual intake, which was captured with a three-day food record (see Appendix 5). Macronutrients were matched by adjusting meal portion sizes and introducing snacks as needed. Closely replicating the habitual diets minimised disruption to participants' daily lives and reduced compliance burden. Participants were also provided one controlled meal immediately post-exercise, which is an important nutrient intake window for muscle recovery.

This study covered key biomarkers associated with pro- and anti-inflammatory response post-exercise. Many studies that investigate inflammation post-exercise typically measure CK (which is more related to muscle damage than inflammation) and often IL-6, but less frequently IL-10 and IL-1β.

This study was open to all genders and included menstruating participants. Limited exercise and performance research is inclusive of genders other than biological males.

In conjunction with the co-researcher Duncan (2024), this project measured many different factors associated with muscle recovery form exercise (muscle damage, inflammation, force changes, soreness and countermovement jump) and presented them into a comprehensive EIMD recovery framework.

4.3 Limitations and Recommendations for Future Research

Firstly, there were limitations with study participation. The sample size was small due to strict exclusion criteria and difficulties recruiting, which has underpowered the study and potentially introduced bias. It was calculated that a minimum of 16 participants per group were needed for the study to reach a clinically significant difference at 80% power and 5% statistical significance using MVIC as the primary outcome based on variations shown in Clifford et al. (2016). However, after 59 expressions of interest (17 vegans and 42 omnivores) only 10 participants completed the study (see Figure 3.1). Eighteen participants were ineligible due to reasons such as hormonal birth control, orthopaedic conditions and being too well-trained, and 19 participants declined to participate. Twenty-one participants were familiarised to the study, however 11 withdrew due to the study time commitment or becoming unreachable. The study concluded with 3 vegans and 7 omnivores having participated in the trial, this inhomogeneous group sizing is also a limitation as it increases variability in the data. The results are not generalisable to broader populations of vegans and omnivores as participants were limited to the age range of 18 – 40 years, Auckland residents only, and people not on hormonal contraception. Future studies with a larger sample size that makes allowances for different ages and contraceptive status would strengthen the results and allow them to be extrapolated to a wider population.

Differences in body composition and fitness levels among participants could also increase results variability. Body composition can affect CK levels as higher muscle mass correlates with higher plasma CKM concentrations (Owens et al., 2019). While this study did not see any differences in CKM values post-exercise, lean muscle mass was measured in preparation, and it is recommended that future studies record lean mass when measuring CKM. While the counter movement jump height was personalised to maximal effort for each participant, the drop-box height was standardised despite differences in participant height and weight. Participants performed the same number of drop-jumps at the same timing intensity, despite varying levels of fitness, which may have created higher skeletal muscle damage in the more sedentary participants. This is due to the 'repeated bout effect' where following an initial bout of eccentric exercise, skeletal muscle adapts and is less susceptible to damage following subsequent bouts of the same exercise (Peake, Suzuki, et al., 2005). Paulsen et al. (2012) recommend classifying participants as low, moderate and high responders to exercise, depending on the severity of their muscle damage, to allow for better interpretation of data. It is suggested that future research considers adopting this classification system.

Variability in dietary intake during the trial may have influenced recovery. While macronutrient and caloric intake were controlled to be comparable across the two groups, micronutrient intake was not and consequently, intake could have varied between participants. The vegan group had significantly lower intake of some micronutrients, and inadequate intake in others. This is of importance as micronutrients such as antioxidants have demonstrated anti-inflammatory properties and inadequate nutrient intake can worsen EIMD and muscle recovery (Milias et al., 2006). Additionally, there were differences in the micronutrient content of the meal provided to participants post-exercise. Macronutrients were matched across the groups, however, the omnivore meal consisted of more vegetables. It is recommended that future research endeavours to guide participants guidelines on at least reaching their minimum micronutrient intake, if not controlling for total amount. Differences in bioavailability of nutrients between animal- and plant-based foods is also a limitation. For example, zinc and iron both have higher bioavailability from animal-based foods

compared to plant-based (Gammoh & Rink, 2017; Hurrell & Egli, 2010). Although this does fall within the study purview of whether nutrient source affects recovery, it is still an important consideration to be mindful of. Additionally, participants completed the habitual food records at their leisure, and therefore, their recorded intake may not have been indicative of typical intake.

Methodological constraints limited the study's comprehensiveness. Inflammatory markers were measured systemically from plasma, instead of locally at the site of injury. This is of relevance as it has been proposed that systemic concentrations may not be an adequate reflection of local inflammation (Clifford et al., 2016). It is suggested for future research if measuring the inflammatory response systemically and not at the site of injury, that more inflammatory biomarkers are measured, such as IL-4 and IL-1ra. Muscle damage was measured only with plasma CKM as an indirect biomarker, however, ultrasound is considered the gold standard in direct measure methods as it can detect the pennation angle as well as fibre damage and oedema (Walker et al., 2004). Hormones such as oestrogen can influence recovery from EIMD (Kendall & Eston, 2002). In effort to limit this confounding factor, menstruating participants commenced the trial within day 1-3 of their cycle, when oestrogen and progesterone levels are typically lowest. However, hormone levels and timing of release vary across individual cycles, therefore serum hormone testing is required for precision. Finally, two researchers conducted the trials, occasionally independently, which may have resulted in inherent differences (test-retest variability) in protocol.

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Appendices

Appendix 1: Recruitment Flyer

VEGE VS MEAT

Ever wondered how muscle recovery post-exercise compares between vegans and omnivores?

Want to know your body composition?

How about a nutrient analysis of what you eat?

JOIN OUR STUDY!

Are you?
Vegan or meat eater for over 2 years, recreationally active or sedentary, and 18-40 years old. Open to any gender!

What will I do?
Leg exercises. Evaluation of leg strength and jump height. Provide blood samples and diet information.

How long will it take?
5 visits to Massey University's Sports Lab in Albany.

What do I get out of it?

- A $50 gift card.
- A report of your body composition.
- Information on how your muscles function and recover.
- Your diet analysed by qualified nutritionists.
- Contact with College of Health researchers.

WANT MORE INFO?

k.vitzel@massey.ac.nz

This project has been reviewed and approved by the Massey University Human Ethics Ohu Matatika 1, Application 0M1 23/06. If you have any concerns about the conduct of this research, please contact A/Prof Louise Brough, Chair, Massey University Human Ethics Ohu Matatika 1, telephone 06 356 9099 x 84575, email humanethics1@massey.ac.nz.

Appendix 2: Participant Information Sheet

PARTICIPANT INFORMATION SHEET

Vegan vs Omnivore Diets - Impact on Muscle Recovery

Researchers: Ben Duncan; Sarah Duncan

Introduction

We are Massey University students studying toward a Master of Science Nutrition and Dietetics, supervised by Associate Professor Andy Foskett and Dr Kaio Vitzel. We are recruiting participants for a project investigating recovery of muscle after exercise, in those consuming a vegan diet (no animal products) or an omnivorous diet (diets that include animal-based foods).

This Participant Information Sheet will help you decide if you'd like to take part in the study. Before you decide, please feel free to talk about the study with other people, such as whānau/family, friends, or healthcare providers. Participation in this study is entirely voluntary and you are free to decline to participate, ask any questions about the study, or to withdraw from the research at any time.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet andthe Consent Form to keep.

Project description

Vegan diets are becoming increasingly popular in New Zealand and around the world. Vegan diets may support good health and may have an impact on sports performance. Recovery is an important consideration for anyone engaged in regular sports or exercise since a fast recovery prevents injury and prepares you for the next training session as soon as possible. However, little is known about how vegan diets affect muscle recovery after exercise, compared to diets that include animal-based foods. Therefore, the main aim of the project is to investigate the impact of a vegan diet vs omnivorous diet on muscle recovery after exercise.

Who can take part?

We are looking for people who:

- Have adhered to either a vegan or an omnivore diet for at least the past 2 years.
- Are recreationally active OR sedentary.
- 18-40 years of age.
- Non-smoker.
- No chronic health conditions (such as diabetes, cardiovascular disease, bleeding disorders, or pulmonary disease).

This study is not suitable for people who:

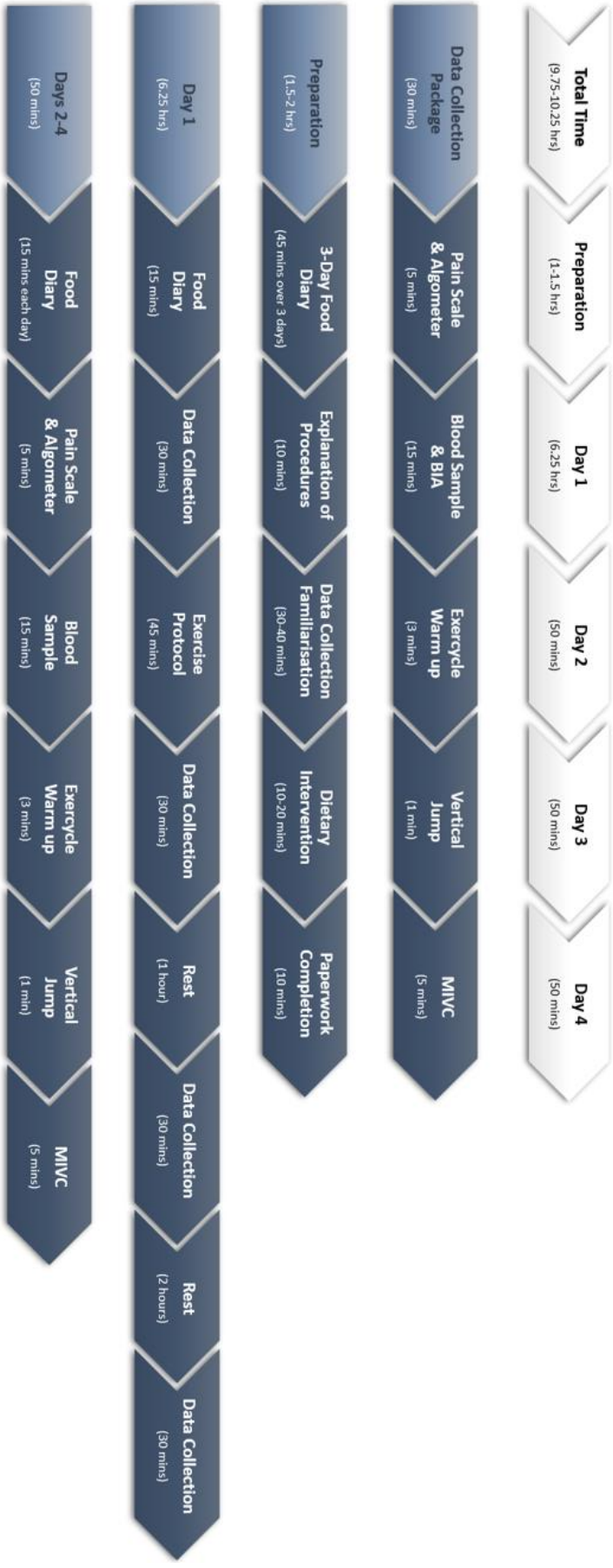
- Are advanced/elite athletes (someone who trains four or more times per week for their sport and competes at a high level)
- Are receiving hormone replacement therapy, or anabolic steroids, or oral contraceptive medication, or have a hormonal implant such as 'the rod' or an IUD (copper IUD is fine).
- Have any conditions identified on the "Health Screening Questionnaire".
- Are amenorrhoeic (do not have periods) for those who have menstrual cycles only.

Project procedures

Before starting the trials, you will be asked to record the food that you eat for 3 days. Since the aim of this project is to compare muscle recovery after exercise between vegans and omnivores, we need to ensure all participants are eating similar amounts of protein and carbohydrate. Your energy intake will be matched to your estimated needs. We will work with you to slightly adjust what you typically eat to meet this target, we will ensure the changes are minimal and not disruptive.

The study requires you to attend 5 sessions at the School of Sport, Exercise and Nutrition at Massey University (Auckland campus). The first session is a familiarisation session. This involves a health-screening questionnaire, consent form and familiarisation with the exercise protocols/tests and other study procedures. The following 4 sessions will comprise the research trials. Please see the below figure for an overview of the procedures and time required. Each session is explained in the paragraphs on the next page.

Estimated time involved



Day 1

Baseline tests

These initial tests will take about 30-40min to complete. We will measure your weight and height, take a blood sample to measure blood markers associated with muscle inflammation, and measure your body composition with a BIA machine (bioelectrical impedance analysis).

To determine how high you can jump, you will be asked to perform 3 vertical jumps on a floor mat. We will also ask how sore your leg muscles feel at different times, using a scale system and a piece of equipment that presses gently on your leg, called an algometer.

Exercise routine

You will be asked to do an exercise routine of 200 drop jumps, which involves jumping off a 0.6m high box and landing with both feet on the ground, followed by an immediate vertical jump up. There will be a 10 second rest after every jump and we will direct you to do this activity safely. You will perform the first 100 drop jumps in 5 sets of 20 jumps with a 2-minute rest between sets. You will then perform the second 100 drop jumps in the same manner. This will take about 45-55 minutes.

We will then repeat the baseline tests as outlined above (excluding the BIA) three more times: immediately after the drop jumps, 1 hour after, and 3 hours after. These tests will take about 20-30 min each time.

Between tests you can rest, so feel free to bring something to occupy your time, such as a laptop or book. You will be provided with a meal to eat during the 1-3 hour test window.

Days 2, 3 and 4

At 24 hours (day 2), 48 hours (day 3) and 72 hours (day 4) after the exercise routine, you willreturn to the lab and repeat the baseline tests as above. This will take about 30 min each time. After day 4, this is the end of the tests. We will also ask you to complete a 4-day food record for everything you eat and drink during the research trial.

Things you will need to avoid during the study

You will need to abstain from alcohol, caffeine, supplements, and hormonal birth control for 24 hours before the first test and until the end of day 4. You will also need to avoid doing any exercise for 48 hours before the start of the study, until after the study has finished.

What are the possible benefits of this study?

You will be involved in an exciting project looking at how vegan and omnivorous diets affect muscle recovery after exercise, and you will gain insight into how research is done. You will receive valuable information about how your body responds to exercise, and you will be contributing to finding answers to unanswered questions about diet and muscle function. We will also provide you with an in-depth nutrient analysis of your diet which will include comparisons to the Ministry of Health recommended guidelines.

What are the possible risks of this study?

You may experience some minor discomfort, such as muscle cramps, delayed muscle soreness, or fatigue, during or after the exercise routine. There is also a chance of soreness, bruising or infection at the injection site when blood samples are taken. We will guide you through how to use the exercise equipment correctly to avoid injury and all practicable steps will be taken to minimize risks. Staff will be fully trained in the procedures and only fully qualified phlebotomists will be taking blood samples. We will also have support staff available in case you do experience any adverse effects.

Will any costs be reimbursed?

Participants will be offered a $50 koha (gift card) to contribute to any transport and food costs incurred on the participation days.

What if something goes wrong?

If you were to be injured in this study, you would be eligible to apply for compensation fromACC just as you would be if you were injured in an accident at work or at home. If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

What will happen to my information?

During this study the researchers will record information about you and your study participation. This includes the results of any study assessments and information collected from you before the study. You cannot take part in this study if you do not consent to the collection of this information.

Identifiable Information

Only researchers will have access to your identifiable information (your name, date of birth).

De-identified (coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report from the study. Instead, you will be identified by a code. Theresults of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and storage of your information

Your information is held at Massy University (Auckland campus) during the study and stored for no longer than five (5) years, then destroyed. All storage will comply with local and/or international data security guidelines.

Who has approved the study?

This project has been reviewed and approved by the Massey University Human Ethics Ohu Matatika 1, Application OM1 23/06. If you have any concerns about the conduct of this research, please

contact A/Prof Louise Brough, Chair, Massey University Human Ethics Ohu Matatika 1, telephone 06 356 9099 x 84575, email humanethics1@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic, and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to,treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred because of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Who do I contact for more information or if I have any concerns?

If you have any questions, concerns or complaints about the study at any stage, you cancontact:

Dr Kaio Vitzel Senior Lecturer

School of Health Sciencesk.vitzel@massey.ac.nz office: (09) 212 7050

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact either of the following people for assistance:

Research coordinators:

Sarah Duncan
MSc Nutrition and Dietetics Student
sduncan2@massey.ac.n

Ben Duncan
MSc Nutrition and Dietetics Student
bduncan@massey.ac.nz

Good Practice and Cultural Safety for Massey University Research

This study has been discussed with Dr Bevan Erueti (Associate Dean Māori, Te Kura Hauora Tangata). We have considered the inclusion of Māori and indigenous values and concepts, allowing for the use of whānau support and appropriate Māori protocols. We acknowledge the concept of manaakitanga, respecting the participant`s inherent dignity and acting in a caring manner towards them by way of:

- Taking full responsibility to perform research in a safe and ethical manner (aroha)
- Providing the participant with all the critical information regarding the study in a clear way, sothey can make informed decisions (tūmanako and whakapono)
- An awareness of the cultural significance and sensitivity for a culturally safe implementation of thestudy (māhaki)
- Respect for the privacy and confidentiality of Māori participants

- Acknowledging the tapu (sacred) nature of blood by offering remaining blood samples (if appropriate) back to the participant

All research activities will adhere to the Covid Protection Framework and guidelines from Ministry of Education and Ministry of Health.

This project has been reviewed and approved by the Massey University Human Ethics Ohu Matatika 1, Application OM1 23/06. If you have any concerns about the conduct of this research, please contact A/Prof Louise Brough, Chair, Massey University Human Ethics Ohu Matatika 1, telephone 06 356 9099 x 84575, email humanethics1@massey.ac.nz.

Participant's Rights

You are under no obligation to accept this invitation, but completion and return of the required form implies consent. If you decide to participate, you have the right to:

- Decline to answer any question
- Ask any questions or withdraw from the study at any time during participation
- Provide information on the understanding that your name will not be used unless you give permission to the researcher
- Be given access to a summary of the project findings when it is concluded.

Appendix 3: Health Screening Questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Vegan diet vs. Omnivorous diet: Impact on recovery of muscle function

Health Screening Questionnaire

Name:_____

Phone: _____

Age: _____

Gender:_____

Please read the following questions carefully. If you have any difficulty, please advise the medical practitioner, nurse or exercise specialist who is conducting the exercise test.

Please answer all of the following questions by ticking only one box for each question:

The questions are based upon the Physical Activity Readiness Questionnaire (PAR-Q), originally devised by the British Columbia Dept of Health (Canada), as revised by [1]Thomas *et al.* (1992) and [2]Cardinal *et al.* (1996), and with added requirements of the Massey University Human Ethics Committee. The information provided by you on this form will be treated with the strictest confidentiality.

Qu 1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Yes ☐ No ☐

Qu 2. Do you feel a pain in your chest when you do physical activity?

Yes ☐ No ☐

Qu 3. In the past month have you had chest pain when you were not doing physical activity?

Yes ☐ No ☐

Qu 4. Do you lose your balance because of dizziness or do you ever lose consciousness?

Yes ☐ No ☐

Qu 5. Have you ever been told that you have high blood pressure?

Yes ☐ No ☐

Qu 6. Do you experience shortness of breath during only mild exertion?

Yes ☐ No ☐

Te Kunenga ki Pūrehuroa

Qu 7. Do you have epilepsy or have you ever had a seizure of any sort? If yes, please explain below.

Yes ☐ No ☐

_____

_____

Qu 8. Are you currently taking any prescribed medication? If so, what?

Yes ☐ No ☐

_____

Qu 9. Are you currently on birth control? If so, what (eg oral, implant, IUD (incl type), injection)?

Yes ☐ No ☐

_____

Qu 10. Do you ever get pains in your calves, buttocks or at the back of your legs during exercise which are not due to soreness or stiffness?

Yes ☐ ☐ No

Qu 11. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

Yes ☐ No ☐

Qu 12. Have you recently undergone surgery or are you carrying an injury? Explain.

Yes ☐ No ☐

_____

_____

_____

Qu 13. Do you smoke?

Yes ☐ No ☐

Qu 14. Do you perform regular leg resistance/strength exercise?

Yes ☐ No ☐

Qu 15. Are you currently ill in any way? Please explain.

Yes ☐ No ☐

_____

_____

_____

Te Kunenga
ki Pūrehuroa

Qu 16. Are you aware of any other reason why you should not participate in physical exercise without medical supervision? If so, what?

Yes ☐ No ☐

_____

_____

_____

Qu 17. Do you have any issues with having your blood taken?

Yes ☐ No ☐

Qu 18. Do you have any bleeding or healing disorders?

Yes ☐ No ☐

Qu 19. Are there any issues that may prevent you from completing approximately 45 minutes of drop jump exercises? If yes, please explain.
(Please refer to the participant information sheet for drop jump protocol.)

Yes ☐ No ☐

_____

_____

_____

I have read, understood and completed this questionnaire.

Signature: _____ Date: _____

References

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Te Kunenga ki Pūrehuroa

Appendix 4: Consent Form

Vegan diet vs Omnivorous diet:

Impact on recovery of muscle function

Consent Form for Study Volunteers

This consent form will be held for a minimum period of five (5) years

I have read the Participant Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I understand that I have the right to withdraw from the study at any time and to decline to answer any questions (if I choose to withdraw, I cannot withdraw my data from the analysis after the data collection has been completed).

I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project.)

I agree to participate in this study under the conditions set out in the Participant Information Sheet.

Signature_____ **Date**_____

Full Name (printed) _____

Phone Number_____

Age _____ **Date of Birth** _____

Appendix 5: Food Diary

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Muscle Recovery Study

3 Day Food Record (Pre-Trial)

Thank you very much for taking part in this study. We are extremely grateful for your time, effort, and commitment.

If you have any questions, please contact Ben Duncan on b.duncan@massey.ac.nz or Sarah Duncan on s.duncan2@massey.ac.nz

All information in this diary will be treated with the strictest confidence. No one outside the study will have access to this.

Study ID: 103

3 Day Food Record - what to do?

- Record all of the food that you eat and drink on **consecutive days for 1 weekend day and 2 week days at your convenience.** For example, Sunday, Monday and Tuesday OR Thursday, Friday and Saturday.
- If possible, record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.
- Include all meals, snacks, and drinks.
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.
- Write down any information that might indicate size or weight of the food to identify the portion size eaten.
- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.
- Use as many pages of the booklet as you need.
- You can also save any packets such as muesli bar wrappers and bring them in with your food diary.
- **Please try to eat as normally as possible.** Don't adjust what you normally eat just because you are keeping a diet record and be honest! This record will give us important information about your diet, and help us create a meal plan that closely matches your typical way of eating.

Describing Food and Drink

Provide as much detail as possible about the type of food eaten. For example, brand names and varieties / types of food.

General description	Food record description
Breakfast example – cereal, milk, sugar	2 Weetbix (Sanitarium) 1 cup So Good unsweetened almond milk 1 tsp Chelsea white sugar
Lunch – Meat Free Bacon Style Rashers sandwich and home-made fries	2 slices of wholegrain bread (Vogels) 2 slices Vegie Delights Meat Free Bacon Style Rashers 25g Zenzo Dairy Free Vegan Cheddar Cheese Alternative 2 tsp Tablelands Dairy Free Buttery Spread

	½ cup fries (home-made, deep fried in Pam's sunflower oil) ½ Tbs vegan aioli (Heinz Mayonnaise Vegan Aioli) Water 1 cup to drink
Dinner – Vegan lentils spaghetti Bolognese	½ cup lentil sauce (see attached recipe) 1 cup spaghetti pasta (Homebrand)
Snacks	Tam & Luke Snack Ball Salted Caramel (2 balls, 28g) 1 small banana 2 Salada crackers with 1 tsp peanut butter 20g Doritos Spicy Sweet Chili Flavoured Tortilla Chips
Milo	I x cup Milo made with plant based Milo powder and 150mls So Good unsweetened almond milk, 100 ml hot water. No sugar

Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled…

General description	**Food record description**
Potatoes	2 medium size potatoes cut in slices and fried in 2tbs canola oil 2 large potatoes with skin (boiled)
Black bean and kumara burger	85g black bean and kumara burger (recipe provided) pan-fried in 2tsp olive oil 85g black bean and kumara burger (recipe provided) oven baked

When using foods that are cooked (eg. pasta, rice, vegetables, etc), please record the **cooked portion** of food.

General description	**Food record description**
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat alternatives	1 cup of cooked lentil sauce or 5 oven baked chicken style strips (Fry's)
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

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Recording the amounts of food you eat

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons, and tablespoons. Eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.
- By weight marked on the packages – e.g. a 425g tin of baked beans, a 32g cereal bar.
- Weighing the food – this is an ideal way to get an accurate idea of the quantity of food eaten, in particular for foods such as meat alternatives, fruits, vegetables and cheese alternatives.
- For bread – describe the size of the slices of bread (e.g. sandwich, medium, toast) – also include brand and variety.
- Using comparisons – e.g. Meat alternative equal to the size of a pack of cards, a scoop of vegan chocolate ice cream equal to the size of a hen's egg.
- If you go out for meals, describe the food eaten in as much detail as possible.
- Use the food record instructions provided to help describe portion sizes.

General description	Food record description
Cheese alternatives	1 heaped tablespoon of grated dairy free cheddar cheese 1 slice dairy free cheddar cheese (8.5 x 2.5 x 2mm) 1 cube dairy free cheddar cheese, match box size

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Example day

Time food was eaten	**Complete description of food** (food and beverage name, brand, variety, preparation method)	**Amount consumed** (units, measures, weight)
7:55am	Sanitarium Weetbix	2 weetbix
" "	So good unsweetened almond milk	150ml
" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Home-made pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 meat free bacon style rashers (zenzo), 1 Tbsp chopped spring onion, 3 Tbsp vegan mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	2 x Lotus Biscoff biscuits
6.00pm	Lasagne	½ cup cooked Sunfed Bull free beef meat alternative mince, 1 cup cooked Budget lasagne shaped pasta, ½ cup homemade (recipe attached) vegan bechamel sauce made with soy milk (So Good, regular), ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp Veesey grated pizza blend cheese
6.30pm	Vegan banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Crave dairy free salted caramel fudge frozen dessert	1/2cup (g) (125g)

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DAY 1 **Date:**_____

Time food was eaten	**Complete description of food** (food and beverage name, brand, variety, preparation method)	**Amount consumed** (units, measures, weight)

Appendix 6: Dietary Analysis

Habitual dietary intake

Table A.6.1: Nutrient analysis of participants' 3-day average habitual intake prior to trial.

Nutrient	RDI[#]	Omnivore (n=7)	Vegan (n=3)	P-value*
Energy (kcal)	N/A	2002.1 ± 197.4	1736.0 ± 525.9	0.476[‡]
Protein (g)	0.84 g/kg	100.7 ± 34.1	66.0 ± 15.7	0.139
Carbohydrate (g)	45-65% of EER	231.1 ± 42.9	201.0 ± 54.7	0.372
Total Fat (g)	20-35% of EER	69.00 ± 18.84	68.17 ± 27.42	0.956
Saturated Fat (g)	<10% of EER	23.69 ± 9.27	14.98 ± 1.88	0.157
Trans Fat (g)	<1% of EER	0.84 ± 0.37	0.41 ± 0.04	0.090
Mono Fat (g)	N/A	26.43 ± 5.37	29.94 ± 14.25	0.567
Poly Fat (g)	N/A	12.55 ± 6.99	14.47 ± 9.71	0.727
Cholesterol (mg)	N/A	287.4 ± 176.1	16.3 ± 5.5	**0.007**
N-3 Fatty Acids (g)	Women: 0.9 g/d Men: 1.6 g/d	1.83 ± 1.69	1.14 ± 0.58	0.520
Fibre (g)	Women: 25 g/d Men: 30 g/d	31.46 ± 13.66	24.78 ± 0.32	0.437
Thiamin (mg)	Women: 1.1 mg/d Men: 1.2 mg/d	1.70 ± 1.31	1.61 ± 0.52	0.913
Riboflavin (mg)	Women: 1.1 mg/d Men: 1.3 mg/d	1.98 ± 1.48	2.45 ± 2.27	0.699
Niacin Equivalent (mg)	Women: 14 mg/d Men: 16 mg/d	41.74 ± 21.31	22.10 ± 9.54	0.174
B6 (mg)	Women & Men: 1.3 mg/d	2.20 ± 1.04	1.40 ± 0.79	0.272
Dietary Folate Equivalents (µg)	Women & Men: 400 µg/d	620.3 ± 274.6	347.0 ± 51.4	**0.040**[‡]
B12 (µg)	Women & Men: 2.4 µg/d	4.03 ± 2.77	3.51 ± 4.25	0.820
Vitamin C (mg)	Women & Men: 45 mg/d	171.71 ± 84.06	52.67 ± 41.00	0.052
Vitamin A Equivalent (µg)	Women: 700 µg/d Men: 900 µg/d	1298.9 ± 1505.9	494.3 ± 350.8	0.401
Vitamin E (mg)	Women: 7 mg/d Men: 10 mg/d	12.99 ± 3.02	13.93 ± 8.10	0.860[‡]
Sodium (mg)	N/A	2050.3 ± 819.9	2577.3 ± 294.7	0.323
Potassium (mg)	Women: 2800 mg/d Men: 3800 mg/d	3557.9 ± 914.0	2844.0 ± 1052.6	0.308
Magnesium (mg)	Women: 310 mg/d Men: 400 mg/d	372.7 ± 136.3	324.7 ± 136.3	0.623
Calcium (mg)	Women & Men: 1000 mg/d	749.3 ± 429.4	705.3 ± 562.9	0.895
Iron (mg)	Women: 18 mg/d Men: 8 mg/d	13.26 ± 5.64	13.50 ± 2.46	0.927[‡]
Zinc (mg)	Women: 8 mg/d Men: 14 mg/d	11.07 ± 2.88	9.80 ± 3.29	0.555

Nutrient	RDI[#]	Omnivore (n=7)	Vegan (n=3)	P-value*
Selenium (µg)	Women: 60 µg/d Men: 70 µg/d	85.1 ± 46.4	40.4 ± 8.1	0.148

Values are mean ± SD. Nutrient reference values are for adults aged 19-50 years.
RDI – recommended daily intake. EER – estimated energy requirements. N/A – not applicable. Mono fat – monounsaturated fat. Poly fat – polyunsaturated fat.
*Significant differences between the two diet groups (P<0.05) (Independent 2-tailed T-test).
‡Welch's T-Test used.
[#]National Health and Medical Research Council, Australian Government Department of Health and Ageing, New Zealand Ministry of Health. (2006). Nutrient reference values for Australia and New Zealand. Canberra: National Health and Medical Research Council.

Intervention dietary intake

Table A.6.2: Nutrient analysis of participants' 4-day dietary intake during trial.

Nutrient	RDI[#]	Omnivore (n=6)	Vegan (n=3)	P-value*
Energy (kcal)	N/A	2319.0 ± 298.5	2320.7 ± 321.4	0.994
Protein (g)	0.84 g/kg	97.14 ± 17.77	89.67 ± 12.42	0.532
Carbohydrate (g)	45-65% of EER	302.0 ± 37.9	296.7 ± 44.2	0.850
Total Fat (g)	20-35% of EER	77.10 ± 17.67	77.67 ± 10.60	0.667
Saturated Fat (g)	<10% of EER	21.97 ± 6.46	16.38 ± 4.61	0.229
Trans Fat (g)	<1% of EER	0.59 ± 0.38	0.23 ± 0.03	0.156
Mono Fat (g)	N/A	34.71 ± 9.31	35.91 ± 7.82	0.854
Poly Fat (g)	N/A	13.83 ± 5.91	17.00 ± 2.58	0.415
Cholesterol (mg)	N/A	238.03 ± 199.70	9.52 ± 2.16	**0.010**
N-3 Fatty Acids (g)	Women: 0.9 g/d Men: 1.6 g/d	1.35 ± 0.85	1.46 ± 0.03	0.829
Fibre (g)	Women: 25 g/d Men: 30 g/d	34.28 ± 12.19	31.84 ± 8.21	0.767
Thiamin (mg)	Women: 1.1 mg/d Men: 1.2 mg/d	2.03 ± 1.20	1.94 ± 0.35	0.907
Riboflavin (mg)	Women: 1.1 mg/d Men: 1.3 mg/d	1.82 ± 0.80	2.34 ± 1.04	0.408
Niacin Equivalent (mg)	Women: 14 mg/d Men: 16 mg/d	40.71 ± 13.06	26.47 ± 2.54	**0.028**[‡]
B6 (mg)	Women & Men: 1.3 mg/d	2.17 ± 0.74	1.40 ± 0.06	**0.032**[‡]
Dietary Folate Equivalents (µg)	Women & Men: 400 µg/d	575.1 ± 185.5	499.7 ± 114.3	0.540
B12 (µg)	Women & Men: 2.4 µg/d	3.86 ± 1.94	3.87 ± 4.01	0.996
Vitamin C (mg)	Women & Men: 45 mg/d	149.71 ± 77.30	79.20 ± 15.14	0.054[‡]
Vitamin A Equivalent (µg)	Women: 700 µg/d Men: 900 µg/d	1192.4 ± 1364.5	843.7 ± 28.7	0.680

Nutrient	RDI[#]	Omnivore (n=6)	Vegan (n=3)	P-value*
Vitamin E (mg)	Women: 7 mg/d Men: 10 mg/d	13.45 ± 6.51	13.30 ± 2.56	0.972
Sodium (mg)	N/A	2140.4 ± 792.6	2930.3 ± 848.3	0.194
Potassium (mg)	Women: 2800 mg/d Men: 3800 mg/d	3550.3 ± 1143.8	3362.0 ± 380.4	0.794
Magnesium (mg)	Women: 310 mg/d Men: 400 mg/d	401.2 ± 134.2	416.0 ± 58.1	0.862
Calcium (mg)	Women & Men: 1000 mg/d	740.9 ± 365.6	869.3 ± 362.6	0.624
Iron (mg)	Women: 18 mg/d Men: 8 mg/d	13.30 ± 3.26	18.18 ± 7.48	0.169
Zinc (mg)	Women: 8 mg/d Men: 14 mg/d	11.89 ± 2.52	11.87 ± 2.55	0.989
Selenium (µg)	Women: 60 µg/d Men: 70 µg/d	78.26 ± 31.59	55.90 ± 9.70	0.129[‡]

One missing omnivore diet record, hence only 6 omnivore participants were valid for analysis.
Values are mean ± SD. Nutrient reference values are for adults aged 19-50 years.
RDI – recommended daily intake. EER – estimated energy requirements. N/A – not applicable. Mono fat – monounsaturated fat. Poly fat – polyunsaturated fat.
*Significant differences between the two diet groups (P<0.05) (Independent 2-tailed T-test)
[‡]Welch's T-Test used.
[#]National Health and Medical Research Council, Australian Government Department of Health and Ageing, New Zealand Ministry of Health. (2006). Nutrient reference values for Australia and New Zealand. Canberra: National Health and Medical Research Council.

Appendix 7: Supplementary Muscle Function Results

The following is an extract from the results section of "Macronutrient source (vegan vs. omnivorous diet): Impact on the recovery of muscle function and performance after damaging exercise" (Duncan, 2024). Measurements were performed simultaneously with this current study, using the same study design, cohort, and protocol.

3.4 Results

3.4.1 Participants

All participants completed all of the muscle function (MIVC, RFD), muscle performance (Jump Height), and subjective muscle soreness (Pain at rest, per muscle group, and during activity) tests. One participant missed the 24 h time point for the PPT muscle soreness measures due to presenting at the lab in inappropriate attire. Participant characteristics are reported in Table 3.1.

Table 3.1: Baseline characteristics of participants by Diet (Vegan or Omnivore)

Variable	Omnivore (n=7)		Vegan (n=3)		t-test (P-value*)
	Male (n=2)	Female (n=5)	Male (n=2)	Female (n=1)	
Age (yr)	25 ± 5.35		32.3 ± 5.77		0.088
Height (cm)	167.9 ± 8.2		173.7 ± 9.5		0.352
Weight (kg)	70.9 ± 8.5		63.4 ± 16.9		0.362
Fat Free Mass (%)	72.1 ± 11.5		81.0 ± 4.9		0.241
Baseline MVC (N)	298.5 ± 132.5		301.7 ± 93.1		0.971
Baseline RFD 25% ($N \cdot s^{-1}$)	1289 ± 672		1123 ± 348		0.702
Baseline RFD 50% ($N \cdot s^{-1}$)	859 ± 448		748 ± 232		0.702
Baseline RFD 75% ($N \cdot s^{-1}$)	430 ± 224		374 ± 116		0.702
Baseline Jump Height (cm)	37.6 ± 8.1		34.3 ± 7.9		0.547
Baseline PPT Rectus Femoris ($kg \cdot cm^2$)	5.4 ± 2.3		3.9 ± 0.6		0.158‡

Baseline PPT Vastus Lateralis (kg·cm^2)	4.4 ± 2.1	3.6 ± 0.4	0.523
Baseline PPT Vastus Medialis (kg·cm^2)	4.3 ± 1.8	3.7 ± 0.9	0.499‡
Baseline PPT Biceps Femoris (kg·cm^2)	4.4 ± 1.6	4.2 ± 0.3	0.790

Values presented as mean ± SD.
*Significant differences between the two diet groups (P<0.05) (Independent t-test).
‡Welch's t-test used.

To account for baseline differences the following variables' data was changed to relative percentage change from baseline: MIVC, RFD25, RFD50, RFD75, PPT Rectus Femoris, PPT Vastus Lateralis, PPT Vastus Medialis, and PPT Biceps Femoris.

3.4.2 Maximal Isometric Voluntary Contraction (MIVC) (N)

There was no significant main effect of diet (2-way ANOVA, $F_{1,8} = 3.319$, $P = 0.106$, $\eta^2 = 0.293$) on MIVC, despite the 'large' effect size observed. However, there was a significant main effect of time on MIVC, also with a 'large' effect size (2-way ANOVA, $F_{2.20,17.59} = 4.376$, $P = 0.026$, $\eta^2 = 0.354$). In the omnivore group, the largest reduction in MIVC force compared to baseline was 12.3% occurring 1-hour post-exercise ($P = 0.56$) and recovered to baseline values 24 hours post-exercise ($P = 1.00$). Whereas, in the vegan group, the largest reduction in MIVC force compared to baseline was 29.5% occurring 24 hours post-exercise ($P = 0.42$) and recovered to baseline values 72 hours post-exercise ($P = 1.00$) (Figure 3.1). There was no significant interaction effect of time x diet (2-way ANOVA, $F_{2.20} = 2.121$, $P = 0.146$, $\eta^2 = 0.210$) on MIVC, despite the 'large' effect size.

Bonferroni post hoc analysis showed that 24 h after exercise, vegans produced significantly lower force than omnivores ($P = 0.04$), experiencing a 29% reduction in MIVC whereas omnivores had returned to baseline MIVC at this time point (Figure 3.1).

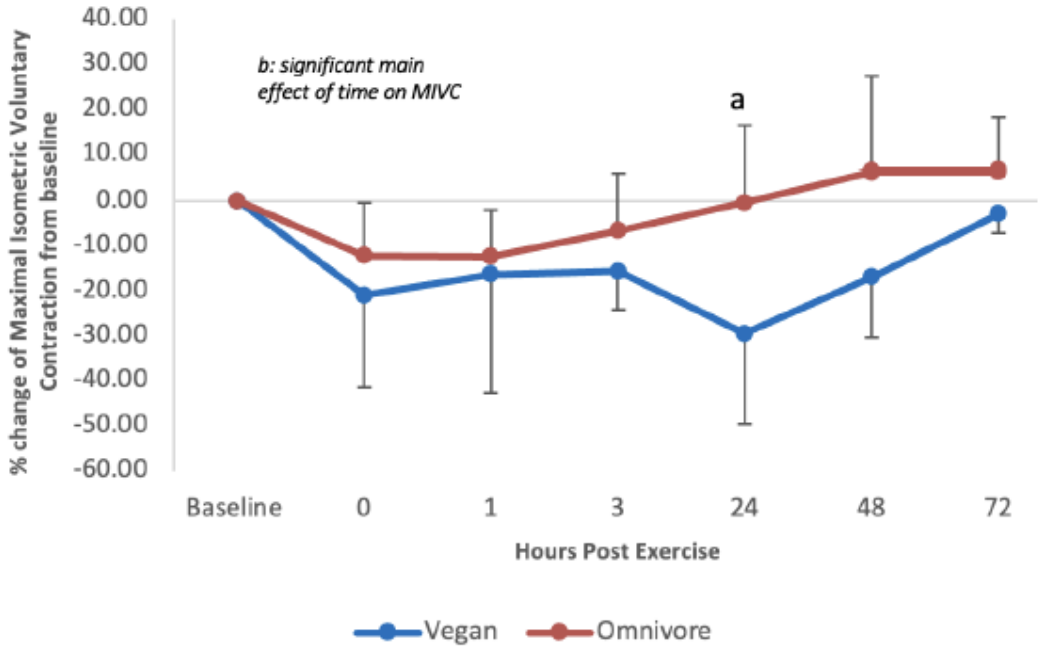

Figure 3.1. % change in MIVC over a 72 h period post muscle-damaging exercise. a: significant difference between diets (P<0.05) (Bonferroni post-hoc analysis); b: significant main effect of time on MIVC (P<0.05) (2-way ANOVA).

3.4.2.1 Rate of Force Development ($N \cdot s^{-1}$)

There was no significant main effect of diet on RFD 25% (2-way ANOVA, $F_{1,8} = 0.774$, $P = 0.405$, $\eta^2 = 0.088$), RFD 50% (2-way ANOVA, $F_{1,8} = 1.068$, $P = 0.332$, $\eta^2 = 0.118$), or RFD 75% (2-way ANOVA, $F_{1,8} = 0.677$, $P = 0.434$, $\eta^2 = 0.078$). However, it is important to note that a 'medium' effect size was observed for diet with the vegan diet group appearing to exhibit a reduced RFD 25%, 50% and 75% as compared to the omnivore diet group.

There was no significant main effect of time on RFD 25%, with only 'small' effect sizes (2-way ANOVA, $F_{2.85,22.76} = 0.319$, $P = 0.801$, $\eta^2 = 0.038$), RFD 50% (2-way ANOVA, $F_{2.69,21.54} = 0.427$, $P = 0.715$, $\eta^2 = 0.051$), or RFD 75% (2-way ANOVA, $F_{2.9,23.2} = 0.328$, $P = 0.798$, $\eta^2 = 0.039$).

There was no significant interaction effect of time x diet on RFD 25% (2-way ANOVA, $F_{2.85} = 0.356$, $P = 0.775$, $\eta^2 = 0.043$), RFD 50% (2-way ANOVA, $F_{2.69} = 0.810$, $P = 0.491$, $\eta^2 = 0.092$, or RFD 75% (2-way ANOVA, $F_{2.9} = 0.320$, $P = 0.804$, $\eta^2 = 0.038$). This was highlighted in the Bonferroni post hoc analysis showing no significant differences occurring between vegans' and omnivores' RFD at all individual time points or between time points within diets.

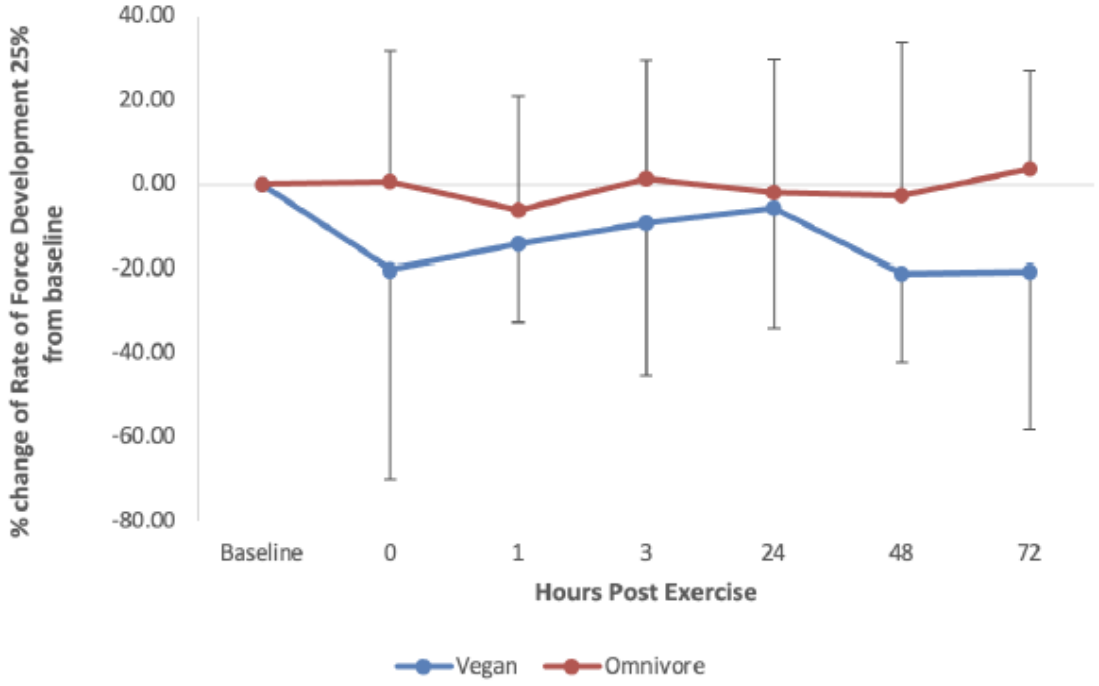

Figure 3.2. % change in RFD 25% over a 72 h period post muscle-damaging exercise.

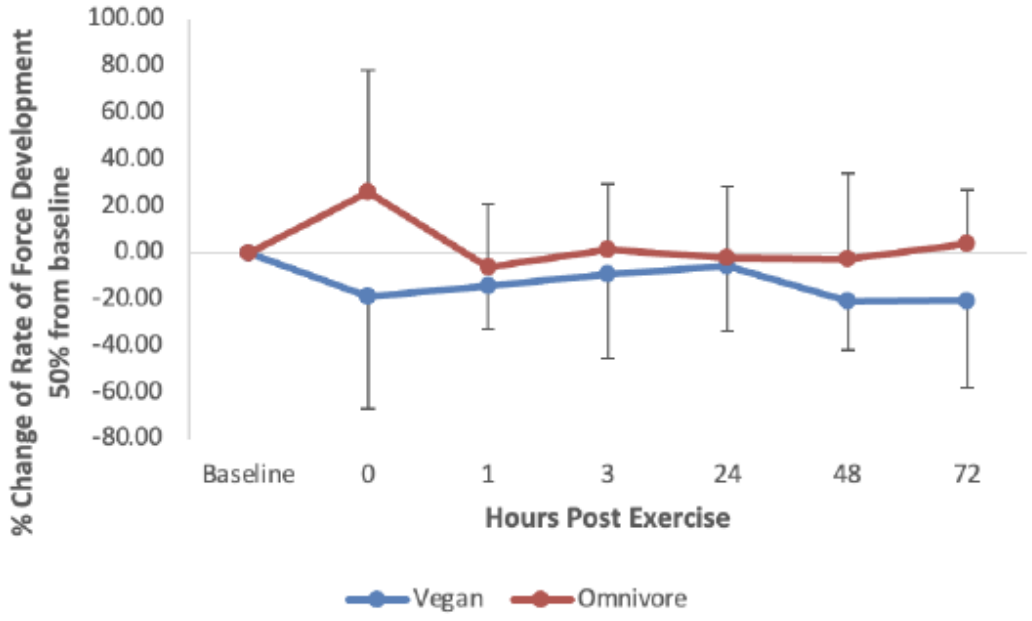

Figure 3.3. % change in RFD 50% over a 72 h period post muscle-damaging exercise.

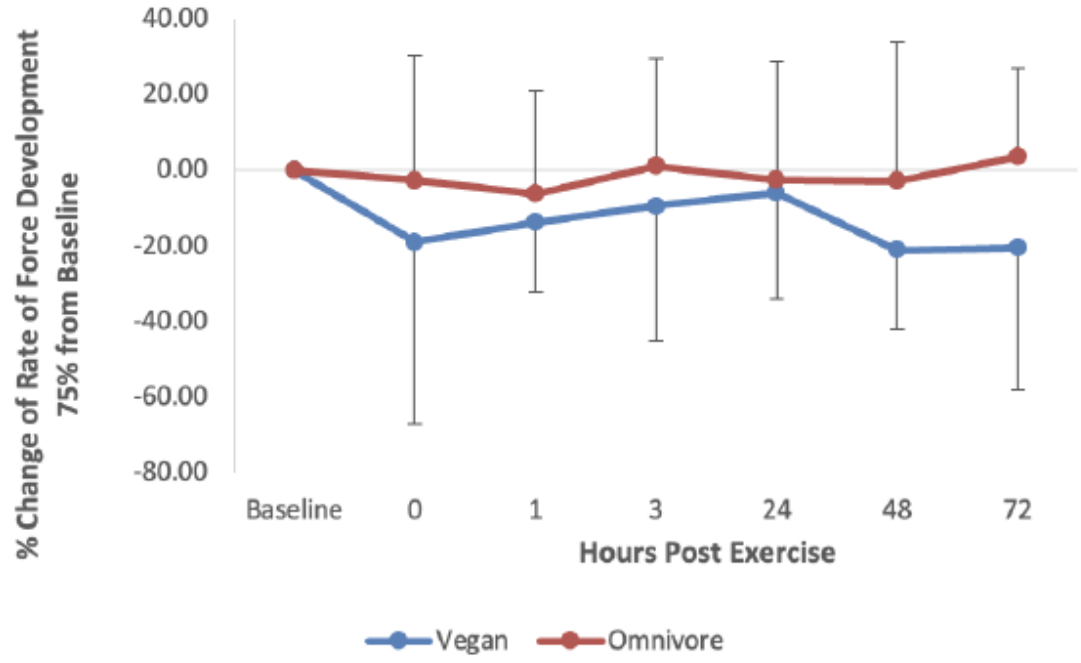

Figure 3.4. % change in RFD 75% over a 72 h period post muscle-damaging exercise.

3.4.3 Jump Height (CMJ)

There was no significant main effect of diet (2-way ANOVA, $F_{1,8} = 0.269$, $P = 0.618$, $\eta^2 = 0.033$) on Jump Height. However, a significant main effect of time (2-way ANOVA, $F_{2.75, 22.02} = 7.409$, $P = 0.002$, $\eta^2 = 0.481$) on Jump Height existed which represented a large effect size. This suggests that the drop jump exercise protocol resulted in jump height loss for participants. In the omnivore group, the most significant reduction in Jump Height compared to baseline occurred 1 hour post-exercise ($P = 0.07$) reducing by 9.3% and recovering to baseline performance 72 hours post-exercise. In the vegan group, the most significant reduction in Jump Height compared to baseline occurred 24 hours post-exercise ($P = 0.72$) reducing by 23.7% and recovering to baseline values 48 hours post-exercise. No significant interaction effect of time x diet (2-way ANOVA, $F_{2.75} = 2.680$, $P = 0.76$, $\eta^2 = 0.251$) on Jump Height was observed.

Bonferroni post hoc analysis showed no significant differences in Jump Height between vegan and omnivore groups at each time point. The largest difference occurred 24 hours post-exercise with Omnivores jumping a mean ± SD of 34.5 cm ± 7.9 cm (i.e., 8.0% lower than baseline) compared to 27.2 cm ± 14.5 cm (i.e., 23.7% lower than baseline) for Vegans, albeit this wasn't statistically significant (P=0.32) (Figure 3.5).

Furthermore, 3 hours post-exercise Omnivores jumped 2.3 cm lower than they did immediately post-exercise (P=0.046) (Figure 3.5).

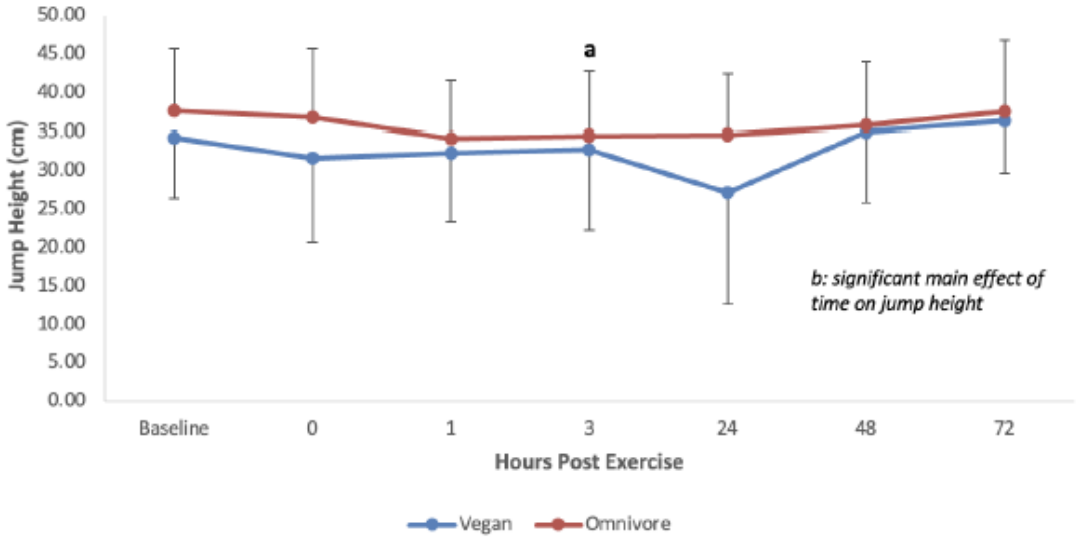

Figure 3.5. Jump Height over a 72 h period post muscle-damaging exercise. a: significant difference compared to 0 h within omnivore diet (P<0.05) (Bonferroni post-hoc analysis); b: significant main effect of time on Jump Height (P<0.05) (2-way ANOVA).

3.4.4 Muscle Soreness

3.4.4.1 Pain Rest - General

There was no significant main effect of diet (2-way ANOVA, $F_{1,8} = 2.543$, $P = 0.15$, $\eta^2 = 0.241$) on Pain at Rest. Albeit, a 'large' effect size was present. A significant main effect of time (2-way ANOVA, $F_{3.89,31.09} = 20.281$, $P < 0.001$, $\eta^2 = 0.717$) on Pain at Rest was observed which represented a large effect size. This highlighted that the damaging exercise protocol resulted in increased pain over time. Also, a significant interaction effect of time x diet (2-way ANOVA, $F_{3.89} = 3.073$, $P < 0.03$, $\eta^2 = 0.278$) on Pain at rest was observed which represented a large effect size.

Bonferroni post-hoc analysis showed that 3 hours post-exercise the pain of the Vegan group was significantly higher than Omnivores ($P=0.02$, $\eta^2 = 0.521$) which represented a large effect size (Figure 3.7).

In the Omnivore group, participants reported significantly greater pain immediately ($P=0.007$), 24 hours ($P=0.002$), 48 hours ($P=0.003$), and 72 hours ($P=0.007$) post-exercise as compared to their pain at baseline. At the 24 h time point, omnivores' soreness peaked and was significantly sorer than 1 hour ($P=0.05$) and 3 hours ($P=0.01$) post-exercise (Figure 3.6).

In the Vegan group, participants reported significantly greater pain 3 hours ($P=0.047$), and 24 hours ($P=0.01$) post-exercise as compared to their pain at baseline. At the 24 h time point, vegans' soreness peaked at which they were significantly sorer than immediately ($P=0.04$), 1 hour ($P=0.02$) and 72 hours ($P=0.01$) post-exercise ((Figure 3.6).

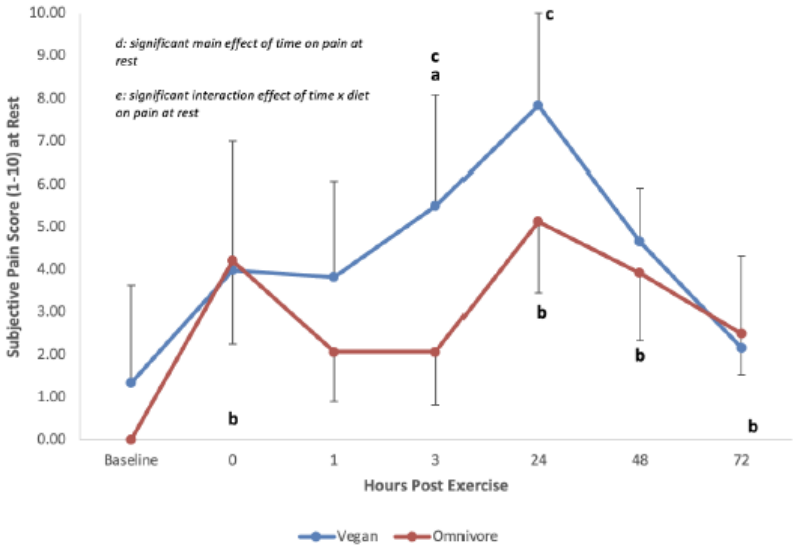

Figure 3.6. Pain at Rest (0-10) over a 72 h period post muscle-damaging exercise. a: significant difference between diets (P<0.05) (Bonferroni post-hoc analysis); b: significant difference compared to baseline within omnivore diet (P<0.05) (Bonferroni post-hoc analysis); c: significant difference compared to baseline within vegan diet (P<0.05) (Bonferroni post-hoc analysis); d: significant main effect of time on Pain at Rest (P<0.05) (2-way ANOVA); e: significant interaction effect of time x diet on Pain at Rest (P<0.05) (2-way ANOVA).

3.4.4.2 Pain Active (CMJ and MIVC)

There was no significant main effect of diet on Pain Active CMJ (2-way ANOVA, $F_{1,8} = 3.387$, $P = 0.103$, $\eta^2 = 0.297$), or Pain Active MIVC (2-way ANOVA, $F_{1,8} = 3.954$, $P = 0.082$, $\eta^2 = 0.331$). However, a 'large' effect size was observed for diet. There was a significant main effect of time on Pain Active CMJ (2-way ANOVA, $F_{3.16,25.25} = 15.749$, $P < 0.001$, $\eta^2 = 0.663$), and Pain Active MIVC (2-way ANOVA, $F_{3.37,26.95} = 13.934$, $P < 0.001$, $\eta^2 = 0.635$) as evident by an increase in pain following the damaging exercise protocol. A 'large' effect size was observed for time. There was no significant interaction effect of time x diet on Pain Active CMJ (2-way ANOVA, $F_{3.16} = 1.991$, $P = 0.138$, $\eta^2 = 0.199$), although there was on Pain Active MIVC (2-way ANOVA, $F_{3.37} = 3.722$, $P = 0.02$, $\eta^2 = 0.318$).

Bonferroni post hoc analysis showed that for both pain during CMJ ($P = 0.015$) and MIVC ($P=0.02$), vegans reported significantly greater soreness 24 hours after exercise compared to omnivores (Figure 3.7, Figure 3.8). Pain during MIVC was also greater after 3 h ($P=0.02$). Vegans peaked soreness during CMJ at 24 h post-exercise which was significantly sorer than baseline ($P=0.04$), 0 h ($P=0.019$), 1 h ($P=0.006$), 3 h ($P=0.006$), and 72 h post-exercise ($P=0.002$). This was also evident with pain during MIVC peaking 24 h post-exercise in vegans which was significantly greater than baseline ($P=0.03$) and 72 h post-exercise ($P=0.005$). Omnivores were significantly sorer during CMJ 24 h ($P=0.003$) and 48 h ($P=0.01$) post-exercise compared to baseline. However, this did not occur during MIVC.

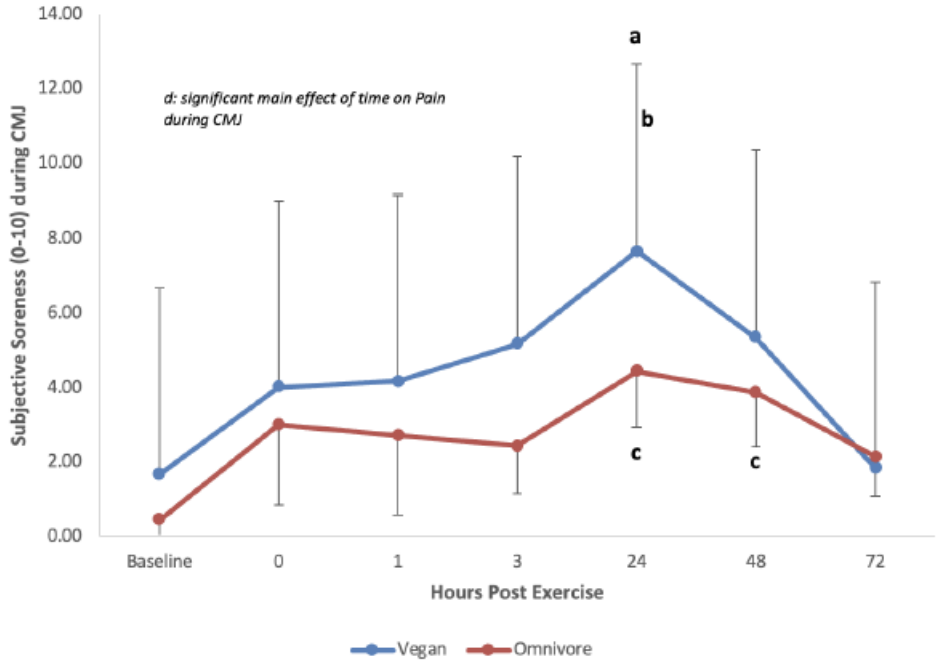

Figure 3.7. Pain during CMJ (0-10) over a 72 h period post muscle-damaging exercise. a: significant difference between diets ($P<0.05$ (Bonferroni post-hoc analysis); b: peak Soreness in Vegans significantly greater than baseline, 0 h, 1 h, 3 h, 72 h ($P<0.05$) (Bonferroni post-hoc analysis); c: significant difference compared to baseline within vegan diet ($P<0.05$). (Bonferroni post-hoc analysis); d: significant main effect of time on Pain during CMJ ($P<0.05$) (2-way ANOVA). CMJ: countermovement jump.

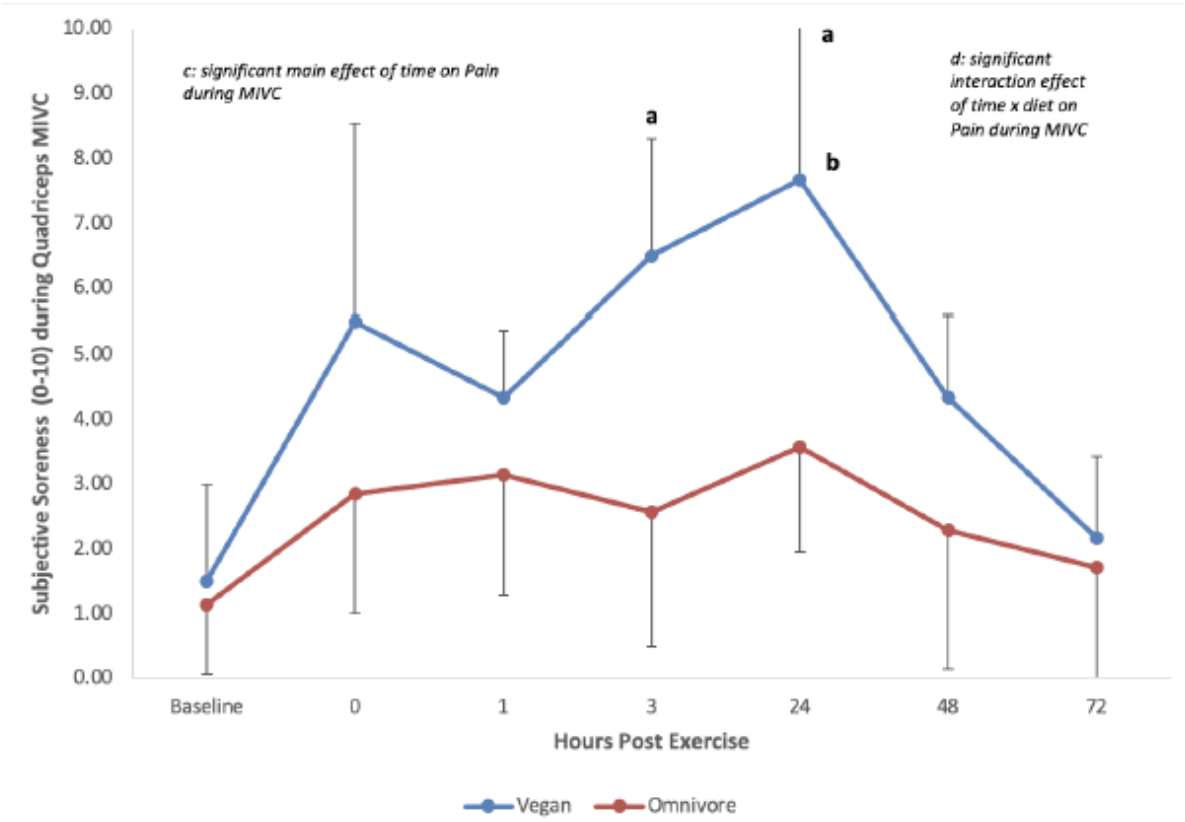

Figure 3.8. Pain during MIVC (0-10) over a 72 h period post muscle-damaging exercise. a: significant difference between diets (P<0.05) (Bonferroni post-hoc analysis); b: peak Soreness in Vegans significantly greater than baseline and 72 h post exercise (P<0.05) (Bonferroni post-hoc analysis); c: significant main effect of time on Pain during MIVC (P<0.05) (2-way ANOVA); d: significant interaction effect of time x diet on Pain during MIVC (P<0.05) (2-way ANOVA). MIVC: maximal isometric voluntary contraction

3.4.4.3 Pressure Pain Threshold (PPT)

There was no statistically significant main effect of diet on PPT Rectus Femoris (2-way ANOVA, $F_{1,7}$ = 1.452, P = 0.267, η^2 = 0.172), PPT Vastus Lateralis (2-way ANOVA, $F_{1,7}$ = 0.790, P = 0.404, η^2 = 0.101), PPT Vastus Medialis (2-way ANOVA, $F_{1,7}$ = 0.820, P = 0.395, η^2 = 0.105), or PPT Biceps Femoris (2-way ANOVA, $F_{1,6}$ = 0.004, P = 0.951, η^2 = 0.001). However, a 'medium' to 'large' effect size was observed for diet in PPT Rectus Femoris, PPT Vastus Lateralis and PPT Vastus Medialis whereas the effect size for diet on PPT Biceps Femoris was 'negligible'.

A significant main effect of time was observed on PPT Rectus Femoris (2-way ANOVA, $F_{2.32,16.21}$ = 3.477, P = 0.05, η^2 = 0.332), PPT Vastus Lateralis (2-way ANOVA, $F_{3.37,23.6}$ = 5.384, P = 0.005, η^2 = 0.435), and PPT Vastus Medialis (2-way ANOVA, $F_{2.69,18.8}$ = 4.680, P = 0.015, η^2 = 0.401) as evident by a reduction in PPT after the damaging exercise protocol. These results displayed a 'large' effect size. While there was no significant main effect of time on PPT Biceps Femoris (2-way ANOVA, $F_{2.67,16.01}$ = 2.831, P = 0.076, η^2 = 0.321), a 'large' effect size was also observed.

Overall, there was no significant interaction effect of time x diet on PPT Rectus Femoris (2-way ANOVA, $F_{2.32}$ = 1.024, P = 0.391, η^2 = 0.128), PPT Vastus Lateralis (2-way ANOVA, $F_{3.37}$ = 0.731, P = 0.559, η^2 = 0.095), PPT Vastus Medialis (2-way ANOVA. $F_{2.69}$ = 1.190, P = 0.337, η^2 = 0.145), or PPT Biceps Femoris (2-way ANOVA, $F_{2.67}$ = 0.927, P = 0.441, η^2 = 0.134).

The Bonferroni post hoc analysis highlighted some differences. Specifically, for PPT of the Rectus Femoris at 1 h post-exercise, omnivores' PPT was 23% lower than that of vegans (P=0.017). Furthermore, omnivores experienced significantly lower PPT at 1 h (P=0.004) and 3 h (P=0.03) post-exercise compared to baseline (Figure 3.9). For PPT of the Vastus Lateralis, omnivores experienced significantly lower PPT at 24 h (P=0.025) and 72 h (P=0.035) post-exercise as compared to baseline (Figure 3.10). For PPT of the Vastus Medialis, omnivores experienced significantly lower PPT at 1 h (P=0.004) and 3 h (P=0.005) post-exercise as compared to baseline. Furthermore, 1 h post-exercise omnivores' PPT of the Vastus Medialis was 17% lower than that of vegans (P=0.035) (Figure 3.11). No significant differences were found for PPT of the Biceps Femoris in the Bonferroni post hoc analysis (Figure 3.12).

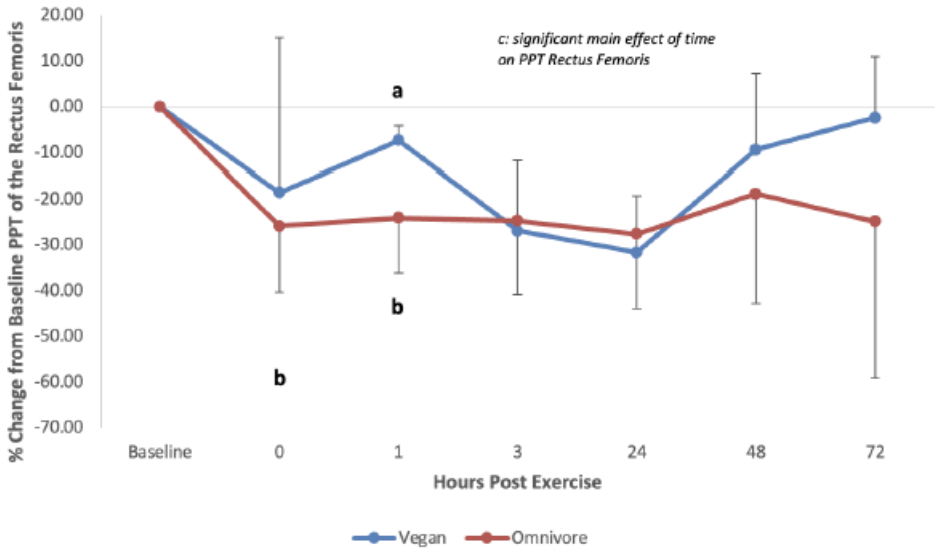

Figure 3.9. % change in PPT Rectus Femoris over a 72 h period post muscle-damaging exercise. a: significant difference between diets (P<0.05) (Bonferroni post-hoc analysis); b: significant difference compared to baseline within omnivore diet (P<0.05) (Bonferroni post-hoc analysis); c: significant main effect of time on PPT of the Rectus Femoris (P<0.05) (2-way ANOVA). PPT: pressure pain threshold.

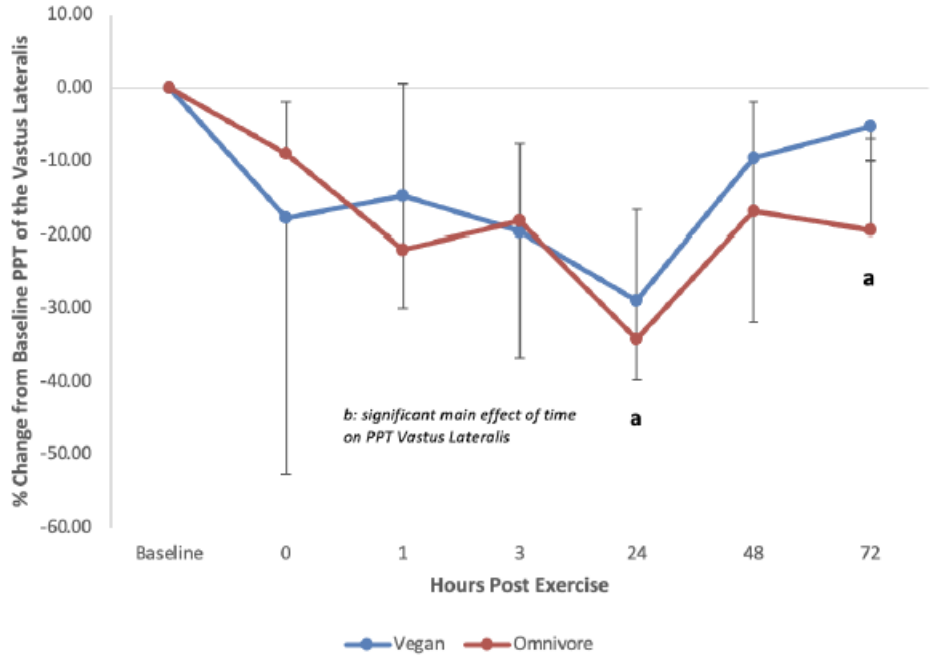

Figure 3.10. % change in PPT Vastus Lateralis over a 72 h period post muscle-damaging exercise. a: significant difference compared to baseline within omnivore diet (P<0.05) (Bonferroni post-hoc analysis); b: significant main effect of time on PPT of the Vastus Lateralis (P<0.05) (2-way ANOVA). PPT: pressure pain threshold.

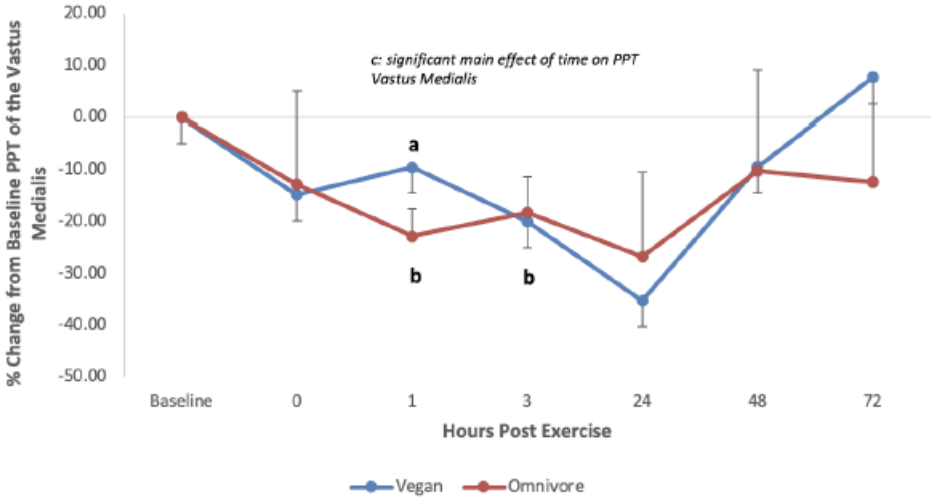

Figure 3.11. % change in PPT Vastus Medialis over a 72 h period post muscle-damaging exercise. a: significant difference between diets (P<0.05) *(Bonferroni post-hoc analysis)*; b: significant difference compared to baseline within omnivore diet (P<0.05) *(Bonferroni post-hoc analysis)*; *c: significant main effect of time on PPT of the Vastus Medialis (P<0.05) (2-way ANOVA). PPT: pressure pain threshold.*

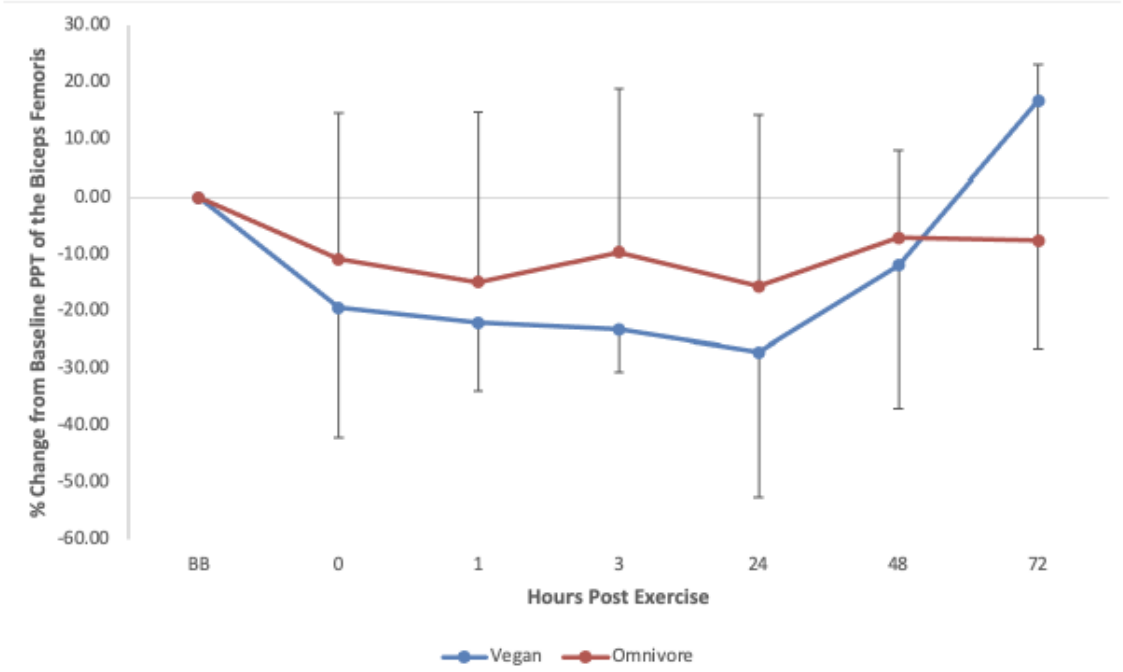

Figure 3.12. % change in PPT Biceps Femoris over a 72 h period post muscle-damaging exercise.

3.2.4.4 Pain by Muscle Group

Significant main effects of time were observed for the left (2-way ANOVA, $F_{2.26,18.08} = 8.214$, $P = 0.002$, $\eta^2 = 0.507$) and right (2-way ANOVA, $F_{2.07,16.56} = 8.052$, $P = 0.003$, $\eta^2 = 0.502$) calf muscles, left (2-way ANOVA, $F_{2.88,23.06} = 12.404$, $P < 0.001$, $\eta^2 = 0.608$) and right (2-way ANOVA, $F_{2.95,23.61} = 11.639$, $P < 0.001$, $\eta^2 = 0.593$) glutes, left (2-way ANOVA, $F_{1.98,15.86} = 13.112$, $P < 0.001$, $\eta^2 = 0.621$) and right (2-way ANOVA, $F_{1.98,15.86} = 13.112$, $P < 0.001$, $\eta^2 = 0.621$) hamstrings, left (2-way ANOVA, $F_{2.45,19.63} = 11.292$, $P < 0.001$, $\eta^2 = 0.585$) and right (2-way ANOVA, $F_{2.5,19.99} = 11.103$, $P < 0.001$, $\eta^2 = 0.581$) inner thighs, and left (2-way ANOVA, $F_{3.35,26.76} = 11.436$, $P < 0.001$, $\eta^2 = 0.588$) and right (2-way ANOVA, $F_{2.95,23.96} = 9.940$, $P = 0.001$, $\eta^2 = 0.554$) outer thighs. No significant main effect of time was found for the left (2-way ANOVA, $F_{2.57,20.59} = 1.261$, $P = 0.311$, $\eta^2 = 0.136$) and right (2-way ANOVA, $F_{2.57,20.59} = 1.261$, $P = 0.311$, $\eta^2 = 0.136$) tibialis.

A significant main effect of diet was observed for the right calf only (2-way ANOVA, $F_{1,8} = 5.948$, $P = 0.041$, $\eta^2 = 0.426$). All other main effects of diet on muscle groups were not significant.

There were also no significant interaction effects of time x diet on any of the muscle groups. However, 'medium' to 'large' effect sizes were evident for all muscle groups suggesting that there is a noticeable to substantial degree of variability associated with the time x diet interactions.

Bonferroni post hoc analysis showed that vegans experienced significantly sorer left (P=0.016) and right (P=0.015) calves, left (P=0.039) and right (P=0.039) inner thighs, and left (P=0.02) and right (P=0.025) outer thighs 3 h post-exercise compared to omnivores.