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Nutrient Source (Vegan vs. Omnivorous Diet): Impact on the Recovery of Muscle Function and Performance after Damaging Exercise

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Science
in
Nutrition and Dietetics

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Abstract

Purpose: This study investigated if the source of nutrients from vegan and omnivorous diets affects the recovery of muscle function and performance following damaging exercise.

Methods: Three (3) vegans (mean age 32.3 ± 5.77 years; 63.4 ± 16.9 kg; 173.7 ± 9.5 cm) and seven (7) omnivores (mean age 25 ± 5.35 years; 70.9 ± 8.5 kg; 167.9 ± 8.2 cm) underwent a muscle-damaging exercise protocol involving 200 drop jumps. At baseline, and 0 h, 1 h, 3 h, 24 h, 48 h, and 72 h post-exercise maximal isometric voluntary contraction (MIVC), rate of force development (RFD), countermovement jump height (CMJ), pressure pain threshold (PPT), pain at rest (overall and by specific muscle), and pain during CMJ and MIVC were measured to assess muscle function, performance and soreness. Participants followed a macronutrient-matched meal plan and consumed a standardised meal post-exercise.

Results: A significant interaction effect of time x diet on Pain Rest, and Pain MIVC existed. Post-hoc analysis found significantly lower MIVC in vegans at 24 h ($P = 0.04$), Pain Rest to be significantly higher in vegans at 3 h ($P = 0.02$), Pain during CMJ ($P = 0.015$) and MIVC ($P = 0.02$) at 24 h was significantly higher in vegans as well as Pain during MIVC at 3 h ($P = 0.02$), PPT Rectus Femoris at 1 h significantly higher in omnivores, and 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 at 3h. A significant main effect of time on MIVC, CMJ, Pain Rest (overall and by muscle), Pain CMJ and MIVC, PPT Rectus Femoris, PPT Vastus Lateralis, and PPT Vastus Medialis. No significant main effects of diet were found. Although, ‘large’ effect sizes existed for many variables. Most nutrients involved in muscle recovery showed no differences between diets other than Vitamin C with near significance ($P = 0.054$). Based on current literature, other nutrients that may have differed but were not quantified in this study were Creatine, L-Carnitine, Vitamin D, Anthocyanins, and Ellagitannins.

Conclusion: It appeared that the omnivore diet group experienced lower reductions in MIVC as compared to the vegan diet group who experienced increased pain at rest (overall and by muscle group) and during activity. The small sample size likely prevented the findings of this study from reaching statistical significance so further, more powerful research addressing this study’s limitations should be performed before recommendations can be made.

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List of Abbreviations

| Abbreviation | Definition |
|---------------------|---|
| AMDR | Acceptable Macronutrient Distribution Range |
| AI | Adequate Intake |
| ADAPT | Adhering to Dietary Approaches for Personal Taste |
| BIA | Bioelectrical Impedance Analysis |
| BCAAs | Branched Chain Amino Acids |
| CVD | Cardiovascular Disease |
| CMJ | Countermovement Jump |
| CK | Creatine Kinase |
| DOMS | Delayed-Onset Muscle Soreness |
| DIT | Diet-Induced Thermogenesis |
| DHA | Docosahexaenoic Acid |
| DPA | Docosapentaenoic Acid |
| EIMD | Exercise-Induced Muscle Damage |
| EPA | Eicosapentaenoic Acid |
| EAR | Estimated Average Requirement |
| EER | Estimated Energy Requirement |
| CKE-C | Excitation-Contraction |
| ECM | Extracellular Matrix |
| FRAP | Ferric Reducing Antioxidant Power |
| G-G | Greenhouse-Geisser |
| IL-1 β | Interleukin-1 β |
| IL-1ra | Interleukin-1 Receptor Antagonists |
| LDH | Lactate Dehydrogenase |
| MDA | Malondialdehyde |
| MIVC | Maximal Isometric Voluntary Contraction |
| MVC | Maximal Voluntary Contraction |
| MPO | Myeloperoxidase |
| NK | Natural Killer |

| | |
|---------------------|---|
| NO | Nitric Oxide |
| Nrf2 | Nuclear Factor Erythroid 2-Related Factor 2 |
| N-3 PUFAs | Omega-3 Polyunsaturated Fatty Acids |
| PCr | Phosphocreatine |
| PUFAs | Polyunsaturated Fatty Acids |
| PPT | Pressure Pain Threshold |
| RFD | Rate of Force Development |
| RONS | Reactive Oxygen and Nitrogen Species |
| RDI | Recommended Daily Intake |
| RMR | Resting Metabolic Rate |
| SERCA | Sarco-Endoplasmic Reticulum Ca ²⁺ Adenosine Triphosphatase |
| SR | Sarcoplasmic Reticulum |
| SFAs | Saturated Fatty Acids |
| SPI | Soy Protein Isolate |
| TNF- α | Tumour Necrosis Factor- α |
| 3-NT | 3-nitrotyrosine |
| 8-OHdG | 8-hydroxy-2'-deoxyguanosine |
| 8-iso-PGF2 α | 8-iso-prostaglandin F2 α |

Chapter 1: Introduction

1.1 Background

Vegetarian, vegan and plant-based diets have existed for many years, across various countries and the respective cultures throughout, however, during the 21st century, interest in these dietary patterns has grown, especially with regard to exercise performance, with the past 5 years showing a high frequency of academic publications (Wirnitzer, 2020). A vegan diet is defined as the exclusive consumption of foods of plant origin (including fungi) and therefore elimination of all foods from animal sources (North et al., 2021). As a result, a vegan diet as compared to an omnivorous diet typically includes higher carbohydrate and dietary fibre intake, lower fat (including lower saturated fatty acids and differing omega-3 fatty acid intakes), protein (including leucine, and L-carnitine), calcium, vitamin D, vitamin B12, zinc and selenium (Clarys et al., 2014; Boutros et al., 2020; Elorinne et al., 2016). Furthermore, a vegan diet contains a negligible amount of creatine compared to an omnivorous diet and thus repletion is dependent on endogenous synthesis (Pohl et al., 2021). When considering foods of note within the Healthy Eating Index-2010 (Guenther et al., 2014) a vegan diet typically contains a greater consumption of fruits and vegetables, and greens and beans as compared to an omnivorous diet (Clarys et al., 2014). As a result of these food choices, a vegan diet will therefore typically contain a greater intake of polyphenols as compared to an omnivorous diet.

In periods of overreaching and overtraining, athletes frequently experience exercise-induced muscle damage (EIMD) (Byrne et al., 2004). Muscle damage is associated with a loss of muscle force and function and ultimately performance, creating concern for an athlete whose goal is to perform (Byrne et al., 2004). It is well-accepted that exercise which involves eccentric muscular contractions (e.g., resistance training, running, high-intensity interval training) results in muscle damage, which is characterized by disruptions in muscular structure, impairment of muscle function, and delayed-onset muscle soreness (DOMS), stiffness and swelling (Byrne et al., 2004). Ultrastructural disruptions in muscle occur as sarcomeres are overstretched leading to what is described as ‘popped sarcomeres’ (Peake et al., 2017). This results in imbalances in calcium homeostasis, inflammation, membrane and excitation-contraction coupling dysfunction and a reduction of muscle strength (Peake et al., 2017). After these

initial events follow a process of muscle fibre degeneration and subsequent regeneration (Clarkson & Sayers, 1999) which coincide with the experience of DOMS, stiffness and swelling (see Chapter 2.7 for further detail on these indirect markers of muscle damage). DOMS comprises muscle tenderness and pain on palpation, and mechanical stiffness (Byrne et al., 2004) which typically presents within approximately 8 hours post-damaging exercise, peaks between 24-48 hours (Clarkson et al., 1992) and subsides by 96 hours (Cleak & Eston, 1992). DOMS should not be used as a sole indicator of muscle damage as muscle function is impaired before soreness arises and muscle damage can become worse following the dissipation of soreness (Byrne et al., 2004; Nosaka & Newton, 2002). As a result, measures of muscle function (e.g., isometric force production via maximal voluntary contraction – see Chapter 2.7) provide a more effective method of measuring muscle damage following eccentric exercise (Warren et al., 1999).

The extent of the loss of muscle function following muscle-damaging exercise is dependent on how trained the damaged muscle is (i.e., the repeated bout effect) (Hyldahl et al., 2017). For example, muscle fibres adapt during the regenerative phase post-EIMD so that future exercise of similar type, intensity and duration results in lower EIMD (Armstrong et al., 1991). In mild cases of EIMD force reduction is less than 20% with full recovery within 48 hours. A force reduction of 20-50% is characteristic of moderate EIMD with a full recovery time of up to 7 days. Any force reduction greater than 50% is considered severe EIMD and can take weeks to return to baseline muscle function (Raastad et al., 2010). Following damaging exercise, the first reduction in force occurs immediately, followed by gradual improvements over 4 hours, and a second reduction at 20-24 hours likely due to additional damage caused by inflammation or central inhibition caused by pain and DOMS (Stožer et al., 2020; Clarkson & Hubal, 2002).

The ability to improve muscle recovery is relevant both during training periods and after athletic competitions. Therefore, nutritional factors that may be implicated in supporting recovery from EIMD and thus of muscle function have been investigated. These include protein and amino acids, polyphenols, omega-3 polyunsaturated fatty acids, Vitamin D, Vitamin C and E, and creatine monohydrate.

Some evidence suggests that when protein or free amino acids are fed before and/or after exercise they can reduce markers of muscle damage and accelerate recovery of force (Buckley et al., 2010; Cockburn

et al., 2010; Nosaka et al., 2006) whereas other evidence finds little to no effect (Wojcik et al., 2001; Pasiakos et al., 2014; Davies et al., 2018). Therefore, although protein and amino acid availability are important for the adaptive remodelling of skeletal muscle after exercise there is insufficient evidence to suggest protein intake after EIMD accelerates the recovery of muscle function (Owens et al., 2018).

Polyphenols such as anthocyanins (found in berries, currents, grapes), quercetin (found in berries, tomatoes, teas, grapes), catechins (found in tea), tart Montmorency cherries, and ellagitannins (found in pomegranates) have been investigated for their potential role in attenuating EIMD due to their established anti-inflammatory and antioxidant properties (Owens et al., 2018) with promising findings (Carey et al., 2021). Despite this, most interventions (McAnulty et al., 2008; Kerksick et al., 2010; Connolly et al., 2006; Trombold et al., 2011) used supplemented forms containing large doses of the polyphenols rather than dietary sources and thus similar effects may not apply to a diet rich in polyphenols, although including a diet high in polyphenols is the current pragmatic suggestion (Owens et al., 2018). Following this, McLeay et al. (2012) demonstrated the beneficial effects of a blueberry smoothie before and after EIMD to increase isometric force recovery, indicating the potential efficacy of polyphenol/antioxidant-rich diets.

Omega-3 polyunsaturated fatty acids (N-3 PUFAs) (found in nuts, seeds, and oily fish) have also been investigated due to their anti-inflammatory properties (Mickleborough, 2013). Supplementation and loading of N-3 PUFAs can reduce markers of muscle damage but fail to attenuate recovery of muscle force (DiLorenzo et al., 2014).

Vitamin D may play a role in modulating the immune response following EIMD with one study (Willis et al., 2012) finding the inflammatory cytokine tumour necrosis factor- α (TNF- α) to be inversely associated with serum 25-hydroxyvitamin D. Another trial found that vitamin D supplementation augmented the recovery of peak isometric force following damaging exercise (Barker et al., 2013).

Vitamin C and E have also been examined due to the theoretical premise that they prevent cellular damage by scavenging free radicals produced after eccentric muscle contractions (Owens et al., 2018). Although, most research examining Vitamin C and E supplementation in EIMD has found little impact on the recovery of muscle function (Thompson et al., 2003) but a potential role in the reduction of DOMS

(Close et al., 2005). As there is no reduction in muscle damage the practical application for recovery of muscle function is lacking and should only be considered in an acute competition setting to reduce muscle soreness and where maximising adaptation is not paramount (Cobley et al., 2011).

Vegan diets contain negligible amounts of creatine compared to an omnivorous diet (Nebl et al, 2019; Pohl et al, 2021). This may be of importance as creatine monohydrate, although in supplement form, has been shown to enhance muscle force recovery after EIMD (Cooke et al., 2009). This may be due to its ability to stabilize dysfunctional cell membranes after damaging exercise as well as modulate the inflammatory response and calcium homeostasis (Bongiovanni et al., 2020).

As illustrated, there is a body of research to suggest that there are various potential nutritional factors involved in the recovery of muscle function. A vegan diet contains less N-3 PUFAs, protein, vitamin D and creatine which all play a potential role in the recovery of muscle function. However, vegan diets typically contain greater amounts of fruit, vegetables, greens and beans which are rich in polyphenols and thus may also have implications for the recovery of muscle function. As a result, the nutritional discrepancies between a vegan diet and an omnivorous diet may result in a differing ability to restore muscle function following damaging exercise.

Studies looking directly at a vegan diet compared to an omnivorous diet on recovery of muscle function are scarce. Research has typically focused on antioxidant status, markers of oxidative stress or whether either of the diets are more favourable to support exercise-induced muscle adaptations (Hevia-Larraín et al., 2021). Nebl et al. (2019) found significant increases in markers of oxidative stress in vegan compared to omnivorous recreational runners while no difference in dietary antioxidant intake was reported. The authors credit this difference to the significantly higher creatine intake of omnivores due to their meat intake and the lack of foods containing creatine in a vegan diet. Vanacore et al. (2018) investigated the oxidative stress status of vegetarians and omnivores. They found that despite the high fruit and vegetable consumption seen in a vegetarian diet which should mitigate oxidative stress, the total antioxidant status of plasma was significantly lower than omnivores and markers of oxidative stress were higher. This was attributed to higher dietary fibre intake in vegetarians which reduces the bioavailability of antioxidant molecules and polyphenols potentially slowing recovery after exercise. Hevia-Larraín et al. (2021) compared a protein-matched vegan and omnivorous diet directly to discover if the source of protein

confers an advantage/disadvantage in supporting exercise-induced muscle adaptations rather than recovery of muscle force. Protein intakes were matched using a soy protein supplement and there were minimal differences in other nutrient intakes, except a higher carbohydrate intake in the vegan group. Under these conditions, there was found to be no difference in muscle adaptations following resistance exercise. Considering recovery of muscle function and markers of muscle damage Saracino et al. (2020) explored if providing a whey-based vs pea and rice-based protein supplement the night before damaging exercise would influence force recovery, inflammatory response, and muscle soreness. Irrespective of the source, protein did not improve any measure of recovery from muscle damage.

No research has looked at whether either diet supports muscle function recovery after damaging exercise. Of the aforementioned research only Saracino et al. (2020) provided standardised meals to participants to meet their energy requirements, although these were all catered for and were not entirely based on participants' habitual intakes. This lowers the ecological validity of the nutritional intervention. Furthermore, there is no evidence to suggest that participants' compliance to these meal plans was measured (e.g., via a food diary). Hevia-Larraín et al. (2021) only matched protein intakes based on habitual intake and topped up with either whey or soy protein supplement to meet their daily $1.6 \text{ g}\cdot\text{kg}^{-1}$ target. This study completed food diaries to determine dietary compliance, although only every 4 weeks over the 12-week intervention. It is important that when controlling nutrition to compare a vegan diet vs an omnivorous diet that all macronutrients and energy intakes are matched, based on participants' habitual dietary intakes and compliance to this throughout the intervention is measured to ensure of ecological validity.

Research comparing these diets is important as the primary goal of a competitive athlete is to perform. In a competition setting an athlete may be less concerned about maximising adaptation following damaging exercise and rather restoring muscle function to be able to perform when need be. Therefore, determining if a vegan or omnivorous diet influences an athlete's ability to do so is of value for future guidance in sports performance recommendations.

1.2 Aim of the Study

The purpose of this study is to determine if the source of nutrient intake, being from the long-term consumption of vegan/plant-based foods vs omnivorous/plant and animal (mixed) foods affects the

recovery of muscle contractile function, soreness and performance following damaging exercise in males and females aged 18-40 years. This study will ensure participants of both groups consume a macronutrient-matched diet to determine if the source of these macronutrients (and therefore the various bioactive compounds within them) has an impact on the recovery of muscle function.

1.3.1 Objectives

1. To determine if recovery of the indirect markers of muscle damage over a 72-hour period following damaging exercise differs in males and females aged 18-40 who consume a vegan vs omnivorous diet.

1.3.2 Hypothesis

Participants consuming a vegan diet compared to an omnivorous diet will demonstrate slower recovery of quadriceps isometric force, jump performance and muscle soreness/pain due to their lower intake of protein (incl. L-carnitine), N-3 PUFAs, vitamin D, and creatine.

1.4 Thesis Structure

This thesis is divided into four chapters and an appendix. Chapter One is an introduction to the background and purpose of the study as well as highlighting the aims, objectives, hypotheses, and researcher's contributions. Chapter Two is a literature review of the most up-to-date and relevant research on the recovery of muscle function following damaging exercise, the nutrients involved in this process and differences between vegan and omnivorous diets. Chapter Three is the research manuscript which includes the abstract, introduction, methods, results, and discussion. Finally, Chapter Four is the conclusion which states how the aims and objectives have been met, discusses how the research will impact sports nutrition and provides recommendations for future directions of research. The appendices include the nutritional intake data, recruitment poster, participant information sheet, informed consent form, health screening questionnaire, Ethics approval letter, food record template, pain scale, muscle sites for pain scale diagram, and recruitment data.

1.5 Researcher contributions

Table 1.1 Summary of researcher’s contributions to the study

| Author | Contribution to Thesis |
|---|--|
| Ben Duncan MSc Nutrition and Dietetics Student | Primary author of the thesis and involved in all study components including assisting with study design, literature review, recruitment, data collection, statistical analysis, and interpretation of results. |
| Associate Professor Andrew Foskett Primary Academic Supervisor | Academic supervisor. Developed the study design. Guided the literature review, statistical analysis, and interpretation of results. |
| Sarah Duncan MSc Nutrition and Dietetics Student | Primary author of individual thesis topic looking at inflammatory markers from the same study. Assisted in study design, recruitment, data collection, statistical analysis, and interpretation of results. |
| Dr Kaio Vitzel Academic Co-Supervisor | Academic co-supervisor. Developed the study design. Guided the literature review, statistical analysis, and interpretation of results. |

Chapter Two: Literature Review

2.0 Introduction

This chapter reviews the current literature on the topic of recovery of muscle function and performance after damaging exercise. It covers what muscle function and performance are, the causes and characteristics of muscle damage, the nutritional factors involved in recovering muscle function and performance, the nutrient discrepancies between a vegan and an omnivorous diet, and the current literature on the impact of vegan vs omnivorous diet on skeletal muscle function. Google Scholar, PubMed and Massey Discover were searched using the various combinations of search terms in Figure 2.1 as well as reference lists of relevant articles.

Date searched: November 2022 – May 2023

Search Criteria/Key Words:

“muscle function” OR “muscle recovery” OR “muscle performance” OR “muscle force” OR “maximal isometric voluntary contraction” OR “MIVC” OR “rate of force development” OR “RFD”

“EIMD” OR “damaging exercise” OR “muscle damage” OR “DOMS” OR “delayed-onset muscle soreness” OR “muscle soreness” OR “muscle pain” OR “eccentric exercise” OR “eccentric contraction” OR “inflammation”

“vegan” OR “plant-based” OR “vegetarian” OR “omnivore” OR “meat-eater”

“protein” OR “nutrient composition” OR “carbohydrate” OR “fat” OR “omega-3” OR “PUFAs” OR “vitamin” OR “mineral” OR “creatine” OR “amino acid” OR “polyphenols” OR “phytonutrients”

“New Zealand”

Electronic Databases: Massey Discover, Google Scholar, PubMed

Figure 2.1. Search Strategy.

2.1 What is muscle function and performance?

Skeletal muscle functions to produce movement, maintain body posture and position, stabilise joints, maintain body temperature and store nutrients, among other metabolic roles (McCuller et al, 2022). One of the main functions of skeletal muscle relevant to exercise performance is its capacity to produce force. The muscle converts chemical energy into mechanical energy to generate this force (McCuller et al, 2022). Fitts et al (1991) suggest that the force-generating capacity of a muscle is determined by numerous factors. For example, muscle and fibre size and length, the angle and physical properties of the fibre-tendon attachment, fibre type, the number of cross-bridges in parallel, the force-velocity relationship, recruitment of motor units, and calcium release/sensitivity.

When muscle function is undisturbed (i.e., an undamaged muscle) it is in an optimal condition to elicit maximal performance. Muscle performance is commonly defined by the muscle's strength, power and endurance capacities (LeBrasseur et al, 2008). Muscle performance can be measured by tests such as a vertical jump using a force-sensitive mat (Vanegas et al, 2021), isokinetic dynamometry, 1-repetition maximum, sprint tests, economy/efficiency tests etc which correlate with muscle strength, power and endurance.

2.2 Exercise-Induced Muscle Damage (EIMD)

Exercise-induced muscle damage (EIMD) occurs in athletes who participate in new or unfamiliar exercises, when learning new techniques, or when they experience an increase in volume or intensity of exercise, often termed “overreaching” (Fatouros & Jamurtas, 2016). EIMD results in a temporarily reduced muscle function and performance but can also lead to various adaptive-remodelling processes which ultimately result in improved muscle function and performance over time (Armstrong et al, 1991).

2.3 Initial Stimulus of Muscle Damage: Mechanical Stress

Eccentric muscular contraction under mechanical loading is well-accepted to cause EIMD (Bryne et al., 2004) (Figure 2.2). Firstly, *in vitro* (Edman et al, 1978) and *in vivo* (Westing et al, 1990) force generation is greater for maximal eccentric vs isometric or concentric actions. Further, motor unit activation is lowest in eccentric contraction at given forces (Kellis & Baltzopoulos, 1998). High force generation with low motor recruitment results in substantial mechanical stress on the involved structures resulting in

muscle damage (Enoka, 1996). Sarcomeres are also stretched further in an eccentric contraction leading to what is described as ‘popped sarcomeres’ (Peake et al, 2017) and therefore muscle damage.

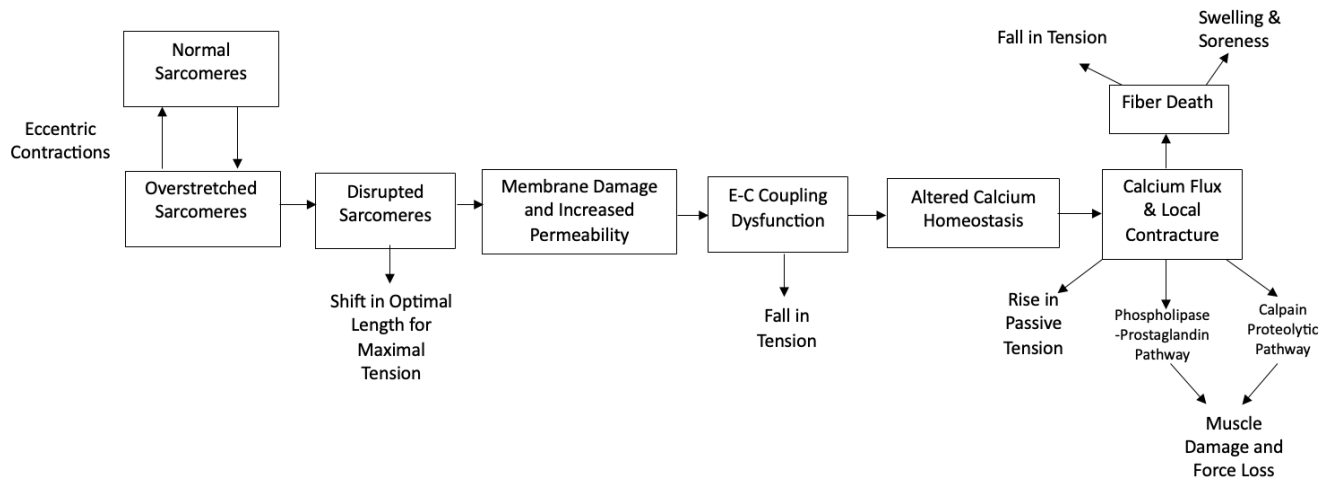


Figure 2.2. Series of Events Leading to Muscle Damage from Eccentric Exercise.

Modified From “Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications”, by U. Proske and D. Morgan, 2001, *The Journal of Physiology*, 537(2), <https://doi.org/10.1111/j.1469-7793.2001.00333.x>

2.3.1 Sarcomere Disruption and Shift in Optimal Length

Figure 2.2 highlights the impact of eccentric contraction on sarcomere disruption. Morgan (1990) showed that during active stretching of the muscle (i.e., the action the muscle is subject to during an eccentric contraction), the weakest half-sarcomere will take up the length change. On the descending limb of the skeletal muscle length-tension curve, these sarcomeres become progressively weaker until they lengthen rapidly to the point of no myofilament overlap. This is the point where the tension in passive structures balances the active tension in contiguous sarcomeres that still have myofilament overlap. This process is continually repeated with the next weakest sarcomere stretching until the end of the stretch where the muscle relaxes and overstretched sarcomeres re-intertwine to continue their normal function (Talbot & Morgan, 1996). Although, during repeated eccentric contraction some sarcomeres become disrupted and fail to do so resulting in a shift in the optimum length of the muscle for maximal tension in the direction of longer muscle lengths (Proske & Morgan, 2001). Adjacent sarcomeres to those disrupted return to a shorter length than before the eccentric contractions and thus the muscle would need to be stretched

further to achieve their optimal tension-length relationship (Proske & Morgan, 2001). As a result, force is lost.

2.3.2 Membrane Damage and Impaired Excitation-Contraction (E-C) Coupling

Myofibrils are connected to the membrane via the dystrophin complex (Gao & McNally, 2015) so when sarcomeres become overstretched this disrupts the anchoring structures resulting in disruption to the T-tubule system and membrane damage (Stožer et al, 2020). An increase in plasma membrane permeability is observed, which disrupts excitation-contraction (E-C) coupling (Macpherson et al, 1996) resulting in a further fall in tension. Although, this is reversible with caffeine due to its ability to open sarcoplasmic reticulum (SR) calcium channels independently of the T-tubule system, thus bypassing the E-C coupling system (Warren et al, 1993).

2.3.3 Calcium Homeostasis

Disruption of the T-tubule system will eventually cause the calcium channels of the SR membrane to become affected (Corona et al, 2010). With damage to plasma and SR membranes, calcium will flux into the sarcoplasm which triggers a local contracture and thus a rise in passive tension and subsequently a loss in active tension (Allen et al, 2005). The elevated calcium concentration activates two pathways that can further contribute to damage and force loss; the phospholipase-prostaglandin pathway (Clarkson & Sayers, 1999) and the calpain proteolytic pathway (Huang & Zhu, 2016). Firstly, the phospholipase-prostaglandin pathway activation results in activated phospholipase A2 which promotes further damage by breaking down the cell membrane and allowing for the loss of intracellular components (McKune et al, 2012). Secondly, the protease calpain 3 is activated with the increased calcium concentration. Calpain 3 plays a role in enzymatic degradation by cleaving Z-disc-associated cytoskeletal proteins such as desmin, vimentin, and α -actinin but sparing myosin and actin as these are not recognised by calpain as substrates (Stožer et al, 2020). This leads to damage in the Z-disc region of the sarcomere.

2.3.4 Extracellular Matrix Disruption

Mechanical eccentric loading has been shown to disrupt the extracellular matrix (ECM) (i.e., the muscle surrounding connective tissue that has linkages to complexes spanning from the membrane) (Stožer et al, 2020). As a result, intracellular reactions occur not only in muscle cells but vascular, inflammatory, stromal and satellite cells too which may lead to the transcription and secretion of signalling molecules

involved in muscle regeneration and adaptation (Fatouros & Jamurtas, 2016). Further, as sarcomeres are overstretched to the point of membrane damage, permeability for various muscular proteins (e.g., creatine kinase (CK) and myoglobin) increases (Sorichter et al, 1999). These can be measured in the plasma as a biomarker for EIMD.

2.4 Initial Stimulus of Muscle Damage: Metabolic Depletion

An alternate hypothesis resulting in EIMD is the metabolic stress model. This suggests that EIMD is the result of metabolic deficiencies in the muscle, or these deficiencies increase the susceptibility to EIMD due to mechanical stress (Tee et al, 2007). Exercise results in a reduction of high-energy phosphates (Tee et al, 2007) which coupled with low glycogen may induce muscle damage (Krustrup et al, 2006; Warhol et al, 1985). During exercise, the action of sarco-endoplasmic reticulum Ca^{2+} adenosine triphosphatase (SERCA) in the sarcolemma is reduced leading to elevated cytosolic Ca^{2+} and a cascade of metabolic effects which lead to muscle damage (Armstrong et al, 1991). Although, this hypothesis is more feasible with EIMD coming from endurance exercise rather than exercise where maximal eccentric contraction is necessary (Stožer et al, 2020).

2.5 Inflammatory and Immune Response to Exercise

Inflammation is a key process involved in muscular repair and regeneration after EIMD (Tidball et al, 2014). Historically, inflammation following EIMD has been viewed as a detrimental process associated with pain, tissue damage and prolonged recovery (Toumi & Best, 2003). Although, the inflammatory response following EIMD is actually a tightly coordinated process which aids the recovery of normal muscle function and is necessary for adaptation (Peake et al, 2017).

2.5.1 Leukocytes

The inflammatory response to eccentric exercise begins immediately with a rise in neutrophils that peaks within 24 hours and may exacerbate muscle damage (Close et al, 2004). This causes fluid, plasma proteins and other inflammatory cells (e.g., natural killer cells, and lymphocytes) to transport into the damaged muscle (Stožer et al, 2020). Neutrophils, which may be attracted to the breakdown products of calpain-mediated cytoskeletal protein degradation (Tidball & Villalta, 2010) also begin clearing necrotic debris in conjunction with macrophages, through phagocytosis. After around 24 hours, macrophages become the predominant leukocyte in the damaged muscle (Peake et al, 2017).

2.5.2 Cytokines

Following eccentric exercise, myocytes and leukocytes produce pro-inflammatory cytokines (e.g., interleukin-1 β : IL-1 β and tumour necrosis factor- α : TNF- α) for up to 5 days (Peake et al, 2015). These cytokines trigger the differentiation of macrophages to an M1 phenotype which produces reactive oxygen and nitrogen species (Tidball & Villalta, 2010) and are involved in exacerbating muscle damage as well as removing debris. Macrophages M1 and M2 are also involved in the production of pro- and then anti-inflammatory cytokines. Reactive oxygen species are generally toxic to cells but may also mediate adaptation to exercise (Crane et al, 2013). Further, TNF- α activates the ubiquitin-proteasome pathway which is involved in muscle remodelling after damaging exercise (Murton et al, 2008). After 2 days, Th2-type (helper T-cells) cytokines (i.e., IL-4, IL-10, IL-14) influence the replacement of M1 macrophages to M2 which leads to the activation of satellite cells involved in repair and remodelling (Tidball & Villalta, 2010).

Furthermore, during exercise muscle cells play a role in attenuating the inflammatory process by producing anti-inflammatory myokines such as IL-1 receptor antagonists (IL-1ra), IL-4, IL-6, and IL-10 (Hennigar et al, 2017; Peake et al, 2005). The extent of myokine production is dependent on the intensity of exercise with higher intensities resulting in greater myokine production (Tee et al, 2007). It is also higher when EIMD occurs from eccentric contraction rather than endurance exercise (Tee et al, 2007).

2.5.3 Leukocyte Accumulation and Muscle Function at Differing Levels of EIMD

The assessment of muscle function by measuring the capacity to produce force is a valid and reliable predictor of muscle damage (Damas et al, 2016). Although, research has also looked at the accumulation of leukocytes in the muscle tissue as a sign of EIMD (Paulsen et al, 2012). Leukocyte accumulation appears to also be dependent on the extent of EIMD. Mild-EIMD (i.e., a decrease in muscle function <20% with full recovery within 2 days) appears to elicit minimal leukocyte accumulation (Peake et al, 2017), although Cramer et al (2007) did find elevated CD68⁺ macrophages after eccentric exercise despite the full recovery of muscle function within 2 days. Moderate-EIMD (i.e., a decrease in muscle function >20% with full recovery within 7 days) appears to result in leukocyte accumulation (Paulsen et al, 2010; Paulsen et al, 2012) although to a greater extent in “high responders” who showed the greatest decrement in muscle function (Paulsen et al, 2010) (noting the subjects denoted as ‘high responders’ were also the most untrained of the subjects). Severe-EIMD (i.e., a decrease in muscle function >50%

with full recovery beyond 7 days) is also consistently associated with leukocyte accumulation with the greatest accumulations being around the same time point as when there were indications of myofiber necrosis (Paulsen et al, 2010).

2.6 Regenerative Phase of Muscle Damage

The regenerative phase following EIMD restores the muscle to its original condition and maximal muscle function is regained. The extracellular matrix (ECM) that is produced during the inflammatory response post-EIMD is remodelled and replaced by regenerated muscle fibres. These muscle fibres arise from the fusion of activated satellite cells with mature fibres and increased protein synthesis. During this process, muscle fibres adapt so that future exercise of similar type, intensity and duration results in lower EIMD (Armstrong et al, 1991) (i.e., the repeated bout effect (Stožer et al, 2020)). This is a process mediated by the resolution of the inflammatory response which is mounted to clear tissue and prevent infection (Chazaud, 2016). If this response is not dampened, healthy tissue may become damaged and muscle recovery will be delayed (Chazaud, 2016).

2.7 Consequences and Markers of EIMD

2.7.1 Maximal Voluntary Contraction Decrements (Force Loss)

Force loss measured by maximal voluntary contraction (MVC) torque is the most reliable indirect predictor of EIMD (Damas et al, 2016; Szczyglowski et al, 2017). When MVC loss is <20% and is fully recovered within 48 hours this is considered mild-EIMD, >20% MVC loss which takes up to 7 days to recover is moderate-EIMD while severe-EIMD results in >50% MVC loss and takes >7 days to recover (Stožer et al, 2020). When observing muscle function following exercise, the first and greatest reduction in force occurs immediately, followed by a gradual improvement over the next 4 hours. At approximately 24 hours force production drops again, likely due to additional damage associated with the signalling pathways elicited by leukocytes and cytokines (e.g., potentially resulting in oxidative stress, proteolysis and inflammation) as well as central inhibition by pain and DOMS (McKune et al, 2012; Close et al, 2004; Stožer et al, 2020; Clarkson & Hubal, 2002). Warren et al (2002) reported that approximately 25% of force loss over the first 3 days following EIMD is a direct consequence of histological damage whereas the remaining 75% loss occurs as a result of the disturbance caused to E-C coupling by mechanically disrupted sarcomeres. By day five reduced force attributed to E-C coupling failure is diminishing and is non-existent by day 14 (Warren et al, 2002). Any additional force loss after 3 days likely occurs due to

the calpain-mediated degradation of cytoskeletal contractile proteins which is seen in moderate-severe EIMD (Warren et al, 2002).

2.7.2 Delayed Onset Muscle Soreness (DOMS)

Delayed onset muscle soreness, termed ‘DOMS’ is a consequence of EIMD which comprises muscle tenderness and pain on palpation, stretch, and contraction as well as mechanical stiffness (Byrne et al, 2004). DOMS typically presents within approximately 8 hours post-damaging exercise, peaks between 24-48 hours (Clarkson et al.,1992) and subsides by 96 hours (Cleak & Eston, 1992). The pain experienced is likely due to prostaglandins, histamine, and bradykinins involved in the mediation of inflammation which sensitise nerve afferents and lower their activation threshold meaning they respond to tissue swelling and histological damage more greatly (MacIntyre et al, 1985; Stožer et al, 2020). It is pertinent to note that, during the initial approximately 8-hour period when an individual experiences an absence of DOMS, the same individual will experience their greatest reduction in force production and is, thus, a poor predictor of EIMD and loss of muscle function (Byrne et al., 2004; Nosaka & Newton, 2002). Further, as the majority of DOMS subsides after 96 hours and loss of muscle function can persist for up to 7 days (or longer in severe circumstances) practical problems can arise if individuals use the dissipation of DOMS as a signal to return to normal training in a potentially compromised state (Byrne et al, 2004).

2.7.3 Swelling

Swelling is the body’s reaction to injury characterised by the increased movement of fluid and leukocytes to injured tissue. Intramuscular swelling and oedema begin to develop within the first hour after exercise and then begin to spread to the subcutaneous space (Clarkson, 1997). Intramuscular swelling can continue to rise and peak within 4-10 days and is experienced alongside DOMS due to the stimulation of sensitised nerve afferents (Stožer et al, 2020). It is difficult to differentiate between swelling and hypertrophy as both processes result in increased muscle size and are measured by using muscle circumference. Although, ultrasonography or magnetic resonance imaging can be used to estimate what the increased muscle size is attributed to (Foley et al, 1999). Due to these difficulties, swelling was not measured in this study as a marker of EIMD.

2.7.4 Biomarkers of Muscle Damage

As membrane permeability increases due to the damaging exercise, this leads to an increase in the plasmatic concentration of various intramuscular molecules, such as myoglobin, creatine kinase (CK), lactate dehydrogenase, myosin heavy chain, skeletal troponin 1, alpha-actin, fatty acid binding protein, aspartate aminotransferase, carbonic anhydrase II isoenzymes, and more (Brentano & Martins Krueel, 2011). Of these biomarkers, creatine kinase is most commonly used to assess EIMD (Stožer et al, 2020) despite its variability between individuals with comparable training status'. There exists a skeletal muscle-specific isoform of CK which differs from isoforms from the heart and/or brain which makes it a valuable biomarker to assess skeletal muscle damage. Plasma creatine kinase only begins to increase after 12 hours post-exercise and peaks at 4-6 days. Therefore, for early assessment of EIMD skeletal troponin 1, alpha-actin, and fatty acid binding protein may be more useful markers (Stožer et al, 2020).

2.8 Nutritional Factors in Functional Recovery of Muscle

2.8.1 Protein and Amino Acids

Protein intake has been extensively researched and is undoubtedly a crucial factor in the maintenance of a positive nitrogen balance, and adaptive remodelling following exercise (Owens et al, 2018). Although, evidence is conflicting as to whether protein and/or amino acids accelerate the recovery of muscle function. Buckley and colleagues (2010) demonstrated that a hydrolysate of whey protein recovered peak isometric torque after eccentric exercise more rapidly than non-hydrolysed whey protein and a flavoured water control. Cockburn et al (2010) assessed the efficacy of a carbohydrate-protein milk-based drink pre or post-exercise on recovery of muscle performance. They highlighted that prior research has demonstrated efficacy but their study used creatine kinase and myoglobin as their measure of EIMD whereas restoration of function is of greater importance for athletes. It was found that consuming the milk-based drink after exercise attenuated decrements in performance and increases in DOMS from EIMD. Both Buckley et al (2010) and Cockburn et al (2010) attributed the mechanism of muscle function restoration to protein's ability to limit ultrastructural damage. Following this, Abbott et al (2019) demonstrated that pre-sleep casein protein accelerates the return of normal muscle function in professional soccer players after an evening match. Despite these studies demonstrating positive effects on muscle function, a recent systematic review (Alcantara et al, 2019) on the acute effect of milk-based beverages (which contain moderate to high amounts of milk-based protein) on muscle recovery was performed. This review looked at team sport athletes and measured muscle function using a variety of

exercise tests (e.g., 1 repetition maximum, time trials, and isokinetic dynamometry) and concluded that more research with more rigorous study designs (such as double-blinded, and randomised sampling to control or intervention group) is needed to determine if milk and its nutrients have a positive effect on muscle function recovery.

As calpain activation after eccentric exercise is involved in damaging sarcomeres and thus reducing force, Kanzaki et al (2019) researched if soy protein isolate ingestion would attenuate this in rats. Rats were fed either a 20% casein or 20% soy protein isolate (SPI) diet for 28 days prior to damaging exercise. The second experiment of this study took half of the SPI rats and provided them with an inhibitor of nitric oxide synthase during the 3 days after the exercise. It was found that soy protein increases nitric oxide (NO) which inhibits eccentric exercise-induced calpain activation and thus aids calcium homeostasis and muscle function recovery. Finally, a recent systematic review and meta-analysis consisting of 8 studies concluded that whey protein has a small to medium impact on recovering force after EIMD although noted that these findings were underpowered (Davies et al, 2018).

Contrary to these data, various evidence has found nil or mixed effects of protein on recovery of muscle function. Wojcik et al (2001) and Coutinho et al (2014) both used carbohydrate-protein beverages to investigate their implications in recovering muscle function which resulted in no differences compared to a placebo. A systematic review by Pasiakos et al (2014) examined the effect of protein supplementation around either cycling, running (shuttle and downhill) or eccentric leg exercise on muscle damage, soreness and recovery in healthy adults between 18-50 years of age. It was found that supplementation after a single bout of exercise did not attenuate markers of EIMD (e.g., CK) and muscle function losses (e.g., time-to-exhaustion, sprint intervals, isometric knee extensor strength, leg flexion torque, and maximal isometric voluntary contraction (MIVC)) although when supplementation continued for extended periods markers of muscle damage and soreness (e.g., subjective soreness using visual analogue scales) were reduced before the subsequent exercise session. Although, these changes were not reflected in improved muscle function and performance.

2.8.1.1 Branched Chain Amino Acids (BCAAs)

The BCAAs leucine, isoleucine and valine are a key substrate for protein synthesis and thus have been studied for their role in muscle recovery from damaging exercise (Howatson et al, 2012).

Supplementation immediately prior to (~5.4-8.3 g) or in the week leading up to (O'Connor et al, 2022) damaging exercise has been shown to reduce biomarkers of muscle damage such as creatine kinase, myoglobin and lactate dehydrogenase (LDH) (Coombes & McNaughton, 2000; Howatson et al, 2012; Kim et al, 2013). Howatson et al (2012) showed that BCAAs supplemented at 10 g twice daily over 12 days around damaging exercise enhances the recovery of maximal voluntary contraction as compared to placebo but this may be attributed to BCAAs' ability to reduce soreness (Jackman et al, 2010). Therefore, as soreness from EIMD increases perceived exertion this may result in reduced muscle performance (Twist & Eston, 2009).

2.8.1.2 Creatine Monohydrate

Creatine is an amino acid which is abundant in muscle and has a wealth of research as an ergogenic aid (Buford et al, 2007) but has also been investigated for its ability to enhance recovery from EIMD (Bongiovanni et al, 2020; Cooke et al, 2009; Northeast & Clifford, 2021). Increasing muscle phosphocreatine stores via supplementation may aid in intramuscular calcium homeostasis (Minajeva et al, 1996), stabilisation of muscle membranes by binding to the cell membrane and reducing fluidity (Saks & Strumia, 1993), reducing inflammation (Bassit et al, 2008) and lessening damage from free radicals (Rahimi, 2011). As exercise increases oxidative stress and inflammation, if creatine can dampen their responses, then this may attenuate EIMD (Northeast & Clifford, 2021). Further, satellite cell generation and myonuclear content may increase in response to creatine supplementation and thus may enhance how quickly muscle fibres are regenerated (Owens et al, 2018). This may explain the improved recovery of force (Cooke et al, 2009; Rosene et al, 2009), reduced muscle soreness (Veggi et al, 2013), and improved range of motion (Veggi et al, 2013) after EIMD with supplementation of creatine. Despite this, various research has shown no benefit on recovery of muscle function and markers of EIMD with creatine supplementation (Boyчук et al, 2016). McKinnon et al (2012) suggested that one explanation for the lack of effect in their study could have been due to using elbow extensor muscles as compared to knee extensor muscles used in studies that did show an effect (e.g., Cooke et al (2009)). Further, the response to creatine supplementation may differ between individuals suggesting there are responders and non-responders (Wax et al, 2021). Work by Syrotuik and Bell (2004) aimed to build a physiological profile of responders and non-responders to creatine supplementation. One determining factor appeared to be the initial intramuscular phosphocreatine (PCr) levels of the individual. Those with the lowest concentrations appeared to be classified as a 'responder'. From this, as participants on vegetarian and

vegan diets consume nearly no exogenous creatine it is likely that they fit into this low concentration and thus ‘responder’ category, highlighting the need for these individuals to supplement creatine. Further, Kaviani et al (2020) highlighted this lower PCr concentration in plasma, serum, red blood cells, and intramuscular but not the brain of vegans/vegetarians as compared to omnivores Finally, Northeast and Clifford (2021) performed a recent systematic review and meta-analysis of 13 studies and concluded that creatine supplementation does not accelerate recovery following EIMD. Although, high and significant heterogeneity was identified for all outcome measures at the various time points post-exercise and more research with greater sample sizes is needed to confirm these conclusions.

2.8.1.3 L-carnitine

L-carnitine is an amino acid derivative endogenously synthesised from the essential amino acids methionine and lysine (Flanagan et al, 2010). Although, under stressful conditions (i.e., EIMD) the body has a greater demand for L-carnitine (O’Connor et al, 2022) and thus supplementation may enhance recovery as deficiency may result in impaired tissue repair (Fielding et al, 2018). L-carnitine can also be obtained from dietary sources such as red meat. Supplementation with L-carnitine for 5 weeks leading up to damaging exercise has been shown to improve restoration of jump performance 48 h after the EIMD (Stefan et al, 2021). As many of these benefits occur as a result of preventing L-carnitine deficiency during exercise-induced stress, supplementation may be beneficial for non-meat-eaters who may struggle to ingest sufficient L-carnitine through dietary sources to meet their increased needs (O’Connor et al, 2022).

2.8.2 Omega-3 Polyunsaturated Fatty Acids (N-3 PUFAs)

Research has examined n-3 PUFAs for their role in reducing inflammation, scavenging reactive oxygen and nitrogen species (RONS), and reducing blood pressure (O’Connor et al, 2022). As a result, there has been interest in their use for recovery from EIMD. Long-term N-3 PUFA (~4 weeks) supplementation may attenuate soreness (Kyriakidou et al, 2021; Mickleborough, 2013) and inflammation (Tartibian et al, 2011) following EIMD but research has failed to show improvements in muscle function recovery (Kyriakidou et al, 2021; Gray et al, 2014; Drager, 2013; DiLorenzo et al, 2014). Despite this, Jakeman et al (2017) demonstrated that acute, high-dose ($1 \text{ g} \cdot 10 \text{ kg bw}^{-1}$) N-3 PUFA supplementation post-damaging drop jumps led to improved recovery of muscle performance determined by squat jump and

countermovement jump. This effect occurred in high eicosapentaenoic acid (EPA) N-3 supplements as compared to high docosahexaenoic acid (DHA).

2.8.3 Vitamin D

Around 5% of New Zealanders are deficient in vitamin D and 27% are below the recommended serum level (University of Otago and Ministry of Health, 2011) which puts these individuals at risk of greater post-exercise muscle damage (Pilch et al, 2020) and inflammation (Barker et al, 2014). Supplementation of 4000 IU vitamin D3 for 4-6 weeks in individuals with varied pre-supplementation vitamin D statuses has been shown to improve the short-term recovery (i.e., immediately to 24 h post-exercise) recovery of muscle force after 100 eccentric-concentric jumps (Owens et al, 2015; Barker et al, 2013). Although, one study by Shanely et al (2014) observed no difference in functional recovery compared to the control. This was due to the lower dosage used ($600 \text{ IU}\cdot\text{d}^{-1}$). Further, this study used portobello mushroom powder as the intervention (i.e., a source of vitamin D2) rather than vitamin D3, and vitamin D2 may be less efficient at raising serum 25-hydroxyvitamin D levels (Tripkovich et al, 2012). Supplementation has also been shown to reduce soreness after aerobic exercise in women with obesity and perceived myalgia as well as in female students 24 h and 48 h after eccentric quadriceps exercise (Vakili et al, 2020; Abdeen et al, 2021). Supplementation also has been shown to reduce markers of muscle damage such as myoglobin, LDH and creatine kinase in healthy, active males after single-leg eccentric-concentric jump exercise and in ultra-marathon runners after eccentric exercise (Barker et al, 2013; Żebrowska et al, 2020), and markers of inflammation and oxidative stress in young adult men after decline treadmill running as well as in male smokers after performing aerobic exercise (Pilch et al, 2020; Vakili et al, 2020; Nikniaz et al, 2021). From this, there is evidence to suggest that vitamin D3 supplementation in doses ranging from 2000 IU to $4000 \text{ IU}\cdot\text{d}^{-1}$, over 4-6 weeks accelerates recovery of muscle function 24 h to 48 h after eccentric exercise in untrained males, especially when individuals are deficient (e.g., during the winter months) (O'Connor et al, 2022). Vitamin D2 does not appear to elicit the same beneficial effects (O'Connor et al, 2022) and thus may cause concern for individuals relying solely on plant sources of vitamin D. This is because vitamin D2 is found in plant sources whereas vitamin D3 predominantly comes from animal sources such as liver, oily fish, beef, and eggs.

2.8.4 Vitamin C and E

Vitamin C has antioxidant properties where it is involved in RONS neutralisation and regeneration of other antioxidant molecules (e.g., vitamin E and glutathione) (Sousa et al, 2014). Vitamin E also has antioxidant properties and can neutralise RONS, as well as inhibit lipid peroxidation (O'Connor et al, 2022). Vitamin C supplementation has been shown to reduce (or delay) markers of muscle damage such as plasmatic creatine kinase (Poulab et al, 2015; Bryer & Goldfarb, 2006) after damaging exercise. Although, this does not appear to correlate with greater recovery of muscle function with some research finding no difference in muscle force recovery compared to placebo when healthy men supplemented with $3 \text{ g}\cdot\text{d}^{-1}$ for 2 weeks prior to and in the 4 days following eccentric exercise (Bryer & Goldfarb, 2006). While others research in healthy adult males found a reduced recovery of muscle function compared to placebo at days 7 and 14 post eccentric exercise (measured via isokinetic dynamometry) with vitamin C supplementation of 1 g 2 hours prior to downhill running and then $1 \text{ g}\cdot\text{d}^{-1}$ for the following 14 days (Close et al, 2006; Thompson et al, 2001). In these studies where recovery was delayed, vitamin C supplementation attenuated increases in ROS and therefore suggests that the ROS produced due to damaging exercise plays a vital role in muscle regeneration and thus recovery of muscle function (Close et al, 2006). Less research has occurred using vitamin E supplementation. Avery et al (2003) supplemented non-resistance trained men with $1200 \text{ IU}\cdot\text{d}^{-1}$ of vitamin E for 3 weeks who then performed 3 resistance exercise sessions each separated by 3 days recovery finding no differences in markers of muscle damage (plasma CK), oxidative stress (plasma and malondialdehyde), and the recovery of muscle function. Jakemanl & Maxwell (1993) had physically active subjects supplement vitamin E ($400 \text{ mg}\cdot\text{d}^{-1}$) for 21 days prior to and 7 days after 60 minutes of box stepping exercise finding no significant differences between vitamin E supplementation and placebo in recovery of MIVC. Furthermore, as these vitamins act on RONS, which are also considered signalling molecules, it is generally considered that supplementation may halt some of the adaptive processes following EIMD, thus reducing the training effect (O'Connor et al, 2022). However, a recent systematic review and meta-analysis by Clifford et al (2020) found that supplementation with these vitamins did not impact aerobic exercise or resistance training adaptations while noting the limited sample size and power of the studies analysed. Although, as vitamin C and E supplementation shows no improvement in muscle function and performance and may be implicated in reducing training adaptations, its use as a recovery strategy should not be recommended.

2.8.5 Polyphenols

2.8.5.1 Anthocyanins

Anthocyanins are rich in fruits and vegetables with red, blue and purple pigments such as berries, currants and grapes. A trial by McLeay et al (2012) showed that when healthy, recreationally trained females consumed a blueberry smoothie (containing 200 g frozen blueberries, 50 g banana, and 200 mL of apple juice) prior to and following EIMD (eccentric quadricep contractions using a dynamometer) this resulted in improved isometric force recovery in the first 36 h following the exercise as compared to placebo. Although force recovery was enhanced, anthocyanin consumption did not attenuate markers of muscle damage (serum CK) or inflammation (IL-6) (McLeay et al, 2012; McAnulty et al, 2011). The researchers suggest that the consumption of blueberries facilitates eccentric exercise-induced anti-inflammatory (via IL-6) events. For example, anti-inflammatory cytokines associated with IL-6 (e.g., IL-10) were not measured but were speculated to have been increased based on work by McAnulty et al (2011) and to have facilitated recovery due to their influence on the replacement of M1 macrophages to M2 which leads to the activation of satellite cells involved in repair and remodelling. McAnulty et al (2011) found that blueberries consumed ($250 \text{ g}\cdot\text{d}^{-1}$) for 6 weeks and 375 g given 1 h prior to 2.5 h of running at 72% $\text{VO}_{2\text{max}}$ in well-trained subjects resulted in significantly lower acute increases in markers of oxidative stress (F_2 -isoprostanes), higher acute increases in anti-inflammatory cytokines (plasma IL-10) and long-term increases in natural killer (NK) cell counts as compared to the control. Furthermore, polyphenols may indirectly increase nitric oxide production by inhibiting enzymes that are associated with its bioavailability (Roelofs et al, 2017). This may explain the increased recovery as nitric oxide has the potential to inhibit m-calpain activity and cytoskeletal proteolysis (Koh & Tidball, 2000; Brandenburg & Giles, 2019). Further research by Hunt et al (2021) looked at anthocyanins coming from blackcurrants by providing $3 \text{ g}\cdot\text{day}^{-1}$ of a blackcurrant extract (providing 105 mg of anthocyanins which equates to approximately 50 g of blackcurrant fresh fruit highlighting the feasibility of this dosage being achieved from dietary sources) to healthy, untrained participants for 8 days prior to and 4 days following concentric and eccentric contractions of the biceps brachii muscle on an isokinetic dynamometer. It was found that consuming anthocyanins resulted in faster force recovery (MIVC), reductions in soreness (visual analogue scale) and blood creatine kinase levels (Hunt et al, 2021). Hunt et al (2021) attributed this faster recovery of muscle function to an increased cellular antioxidant defence capacity through the activation of cellular redox-sensitive signalling pathways (such as nuclear factor erythroid 2-related

factor 2 (Nrf2)/ARE transcription) which results in the upregulation of this antioxidant defence capacity thus lowering oxidative stress during exercise recovery.

2.8.5.2 Ellagitannins

Pomegranate is a rich source of ellagitannins which act as an antioxidant and can ameliorate markers of oxidative stress post-exercise (O'Connor et al, 2022). Improved recovery of muscle force has been shown with pomegranate supplementation in the 3-5 days prior to and 4 days following eccentric exercise (isokinetic dynamometer) in both untrained (Trombold et al, 2010; Machin et al, 2014) and trained males (8 days prior to and 7 days following eccentric exercise) (Trombold et al, 2011). Similar to anthocyanins, Roelofs et al (2017) examined the consumption of ellagitannins (pomegranate extract 1000 mg) before exercise (repeated sprint ability and repetitions to fatigue on the bench press and leg press) and determined it is involved in increasing serum nitric oxide levels (indirectly via increased bioavailability) which results in the potential to inhibit m-calpain activity and cytoskeletal proteolysis (Koh & Tidball, 2000), reducing muscle damage.

2.8.5.3 Tart Montmorency Cherry

A rich source of anthocyanins and quercetin, tart cherry possesses anti-inflammatory and antioxidant properties (Brown et al, 2019) and has been investigated for its ability to improve recovery from EIMD. In the immediate hours and day's following damaging exercise, a reduced decrement of muscle function and performance (e.g., isometric elbow flexion strength, quadricep MIVC, 6-second peak cycling power, 20 m sprint, countermovement jump height (CMJ)) has consistently been shown with the consumption of tart cherries in a range of individuals (trained, untrained, male, female) across a variety of exercise activities (resistance training, sprinting, endurance training) (Connolly et al, 2006; Bell et al, 2015; Bell et al, 2016; O'Connor et al, 2022).

2.8.6 Caffeine

Caffeine has been investigated as an ergogenic aid to sports performance with great efficacy (Harty et al, 2019) but in addition to this, evidence suggests it may play a role in attenuating EIMD (Harty et al, 2019). It has been hypothesised that caffeine may reduce delayed-onset muscle soreness (DOMS), attenuate temporary losses in strength, and reduce markers of muscle damage (Caldas et al, 2022). A recent systematic review by Caldas et al (2022) observed in four studies that caffeine ingestion between 24 h

and 72 h after muscle damage attenuates the perception of pain by up to 26%. They found that caffeine ingestion prior to damaging exercise did not alter creatine kinase after exercise and that, if caffeine was ingested post-exercise, changes to force recovery were inconclusive. Although, muscle force experiences a second reduction at ~24 h post damaging exercise due to central inhibition by pain and DOMS. Therefore, theoretically, if caffeine reduces the perception of pain this may, in turn, improve force recovery.

2.9 Nutrient Discrepancies between Vegan and Omnivorous Diets

The vegan diet excludes all foods of animal origin which creates discrepancies in the nutritional intake of a vegan compared to an omnivore. Karlsen et al (2019) performed a theoretical analysis of the nutritional intake of a vegan diet based on a 30-day meal plan developed from recipes that were said to be popularly used by vegans in the Adhering to Dietary Approaches for Personal Taste (ADAPT) Feasibility Survey (Karlsen et al, 2018). This theoretical intake was then compared to current dietary recommendations.

When examining food group servings, the theoretical vegan diet's total vegetable intake was 180% greater than guidelines, legume intake was 460% greater, whole fruits intake was 100% greater, nut and seed intake was 200% greater, whole grains were 132% greater, dairy intake was 90% lower (noting this contained plant-based milk intake), and, as expected, eggs, poultry, seafood and red meat intake was 100% less than the recommendations (Karlsen et al, 2019).

When considering macronutrient intake, the theoretical vegan diet contains a lower proportion of energy coming from fat (including saturated and unsaturated fatty acids) and protein, with this energy made up through the greater intake of carbohydrate (and dietary fibre) while consuming less added sugars (Karlsen et al, 2019). Micronutrient intake showed lower dietary vitamin D, vitamin B12, and calcium from the vegan diet with dietary vitamin E, vitamin C, folate and magnesium being higher (Karlsen et al, 2019).

2.9.1 Protein Intakes

As well as the theoretical (Karlsen et al, 2019) protein intake of vegans being lower than omnivores, numerous studies have found this to be evident in their actual intake (Clarys et al, 2014; Boutros et al, 2020; Elorinne et al, 2016; Bakaloudi et al, 2021). Despite protein intakes being consistently lower, the

percentage of energy coming from protein is typically 13-15% (Bakaloudi et al, 2021) which is within the Acceptable Macronutrient Distribution Range (AMDR) of 10-35% (Trumbo et al, 2002), albeit at the lower limit. Mariotti and Gardner (2019) discovered using data from the EPIC-Oxford study (Davey et al, 2003; Sobiecki et al, 2016) that the average protein intake of vegans in $\text{g}\cdot\text{kg}^{-1}$ is $0.99 \text{ g}\cdot\text{kg}^{-1}$ as compared to $1.28 \text{ g}\cdot\text{kg}^{-1}$ in their meat-eating counterparts. This is greater than the estimated average requirement of $0.68 \text{ g}\cdot\text{kg}^{-1}$ for males and $0.60 \text{ g}\cdot\text{kg}^{-1}$ for females (National Health and Medical Research Council et al, 2006).

When considering the amino acid adequacy of a vegan diet, Mariotti and Gardner (2019) noted that all 20 amino acids can be obtained from a plant-based diet and although their proportions may not be optimal (e.g., of lysine, methionine, and cysteine), the relatively high total protein intake should lead to amino acid adequacy. Furthermore, there is currently insufficient evidence to suggest that there is a large enough difference in the protein digestibility and bioavailability of plant-based proteins to cause amino acid inadequacy (Mariotti & Gardner, 2019). Although the risk is higher for individuals on an exclusively plant-based diet compared to vegetarians (Mariotti & Gardner, 2019), with plasma concentrations of tyrosine, lysine, methionine and tryptophan being lowest in vegan diets compared to other diet types (Schmidt et al, 2016).

2.9.2 Carbohydrate and Dietary Fibre Intakes

As theoretically predicted (Karlsen et al, 2019), a vegan diet is characterised by a high intake of carbohydrates and dietary fibre (Bakaloudi et al, 2021). The EPIC-Oxford cohort (Davey et al, 2003) demonstrated that male and female omnivores consume 47% and 48% respectively of energy from carbohydrates whereas male and female vegans consume 55% and 56%. Both of these values fall within the AMDR of 45-65% (Trumbo et al, 2002), yet vegans tend to consume a greater percentage of energy as carbohydrates. Clarys et al (2014) suggested a positive association between dietary restriction and the percentage of energy coming from carbohydrates.

2.9.3 Fats

Fat intake as a percentage of energy appears to not significantly differ between vegans (28%) and omnivores (32%) (Davey et al, 2003), although vegans tend to have a lower intake (Bakaloudi et al, 2021). These values both fall within the AMDR of 20-35% of energy from fat (Trumbo et al, 2002).

Although, there are significant differences in the types of fat consumed. Omnivores consume greater saturated fatty acids (SFAs) whereas vegans make up most of their fat intake from polyunsaturated fatty acids (PUFAs) (Bakaloudi et al, 2021). As a result, vegans tend to consume greater omega-3 and omega-6 (linoleic acid) PUFAs. Allès et al (2017) showed vegan's omega-3 intake to be $1.7 \text{ g}\cdot\text{d}^{-1}$ vs $1.3 \text{ g}\cdot\text{d}^{-1}$ of omnivores and omega-6 intake to be $15 \text{ g}\cdot\text{d}^{-1}$ vs $9.2 \text{ g}\cdot\text{d}^{-1}$ which was significantly greater. These respective intakes of PUFAs all meet the adequate intake (AI) (National Health and Medical Research Council et al, 2006) for linoleic acid ($8 \text{ g}\cdot\text{d}^{-1}$ for women and $13 \text{ g}\cdot\text{d}^{-1}$ for men) (found in e.g., seed oils) and α -linolenic acid ($0.8 \text{ g}\cdot\text{d}^{-1}$ for women and $1.3 \text{ g}\cdot\text{d}^{-1}$ for men) (found in e.g., legumes, linseeds, canola oil and margarine, walnuts). Although, as vegans do not consume oily fish they do not meet the AI ($90 \text{ mg}\cdot\text{d}^{-1}$ for women and $160 \text{ mg}\cdot\text{d}^{-1}$ for men) for long-chain PUFAs (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA)). This is reflected in their serum EPA and DHA levels which are lower than omnivores (Elorinne et al, 2016). Although, it is important to note that EPA can be synthesised endogenously from α -linolenic acid which may explain why the serum level of EPA in vegans, although low, was not as low as expected (Elorinne et al, 2016).

2.9.4 Vitamin D

Vitamin D levels can generally be maintained through regular, direct exposure to sunlight and therefore under these circumstances is not required in the diet (Rasmussen et al, 2000). Although, some individuals do not receive adequate sunlight, especially in the winter months and therefore the risk of vitamin D inadequacy or deficiency increases. This is why the AI is set at $5.0 \mu\text{g}\cdot\text{d}^{-1}$ for adults aged 19-50 years (National Health and Medical Research Council et al, 2006). Most foods that contain vitamin D come from animals (except mushrooms) thus putting vegans at risk of not meeting this AI without the consumption of fortified products (e.g., plant milk). Data consistently show a lower vitamin D intake in vegans compared to omnivores (Bakaloudi et al, 2021; Davey et al, 2003; Allès et al, 2017). This is reflected in the lower serum total vitamin D (i.e., 25-hydroxyvitamin D2 and D3) concentrations in vegans as compared to omnivores and the higher likelihood of vegans showing inadequacies (Elorinne et al, 2016).

2.9.5 Other nutrients: Vitamin B12, Calcium, Zinc, and Selenium

Vitamin B12 intake in vegans is limited with most research showing intakes of $0 - 0.9 \mu\text{g}\cdot\text{d}^{-1}$ (Bakaloudi et al, 2021) unless supplementing or consuming fortified foods where if so, the estimated average

requirement (EAR) was reached. Although, one study by Allès et al (2017) found vegans' B12 intakes to be $2.7 \mu\text{g}\cdot\text{d}^{-1}$ which meets the estimated average requirement (EAR) while also reporting that vegans demonstrated the greatest prevalence of dietary B12 inadequacy. Most research (Bakaloudi et al, 2021) shows that an omnivore's B12 intake is adequate. Vegans have also been shown to have the lowest intake of minerals such as calcium, zinc and selenium (Bakaloudi et al, 2021).

2.9.6 Creatine

Endogenous production of creatine provides about 1 g while another approximately 1 g is provided exogenously from animal tissues (e.g., meat, fish and poultry) (Kaviani et al, 2020). Plant-based sources of creatine are nearly nil with any food source containing creatine containing only trace amounts (Balestrino & Adriano, 2019). As a result, vegans consume less creatine than omnivores (Solis et al, 2014). This results in lower muscle and serum creatine levels in vegans compared to omnivores (Kaviani et al, 2020). Furthermore, vitamin B12 deficiency (which is of greater risk in vegans) can impair methionine production which is involved in the endogenous synthesis of creatine (Mahmood, 2014). This contributes to the discrepancies in creatine concentration in various body stores in vegans vs omnivores.

2.10 Vegan vs Omnivore Muscle Recovery

As aforementioned, various nutrients play a role in recovery following EIMD and some of these nutrients are consumed in differing quantities between vegans and omnivores. To date, no research has looked specifically at whether the nutrients coming from a vegan diet as compared to an omnivorous diet affect the recovery of muscle function and performance after EIMD. The only literature considering vegans vs omnivores examined markers of oxidative stress, exercise-induced muscular adaptations and performance, and plant vs animal-based protein supplementation. This literature is highlighted below.

2.10.1 Markers of oxidative stress

It would be expected that, due to the increased fruit and vegetable intake of vegans compared to omnivores (Clarys et al., 2014), vegans would exhibit a more favourable oxidative stress status. Although, the data are mixed. Vanacore et al (2018) collected twelve-hour fasting samples from healthy, sedentary vegan and omnivorous males and found that the total antioxidant status of plasma (measured by ferric reducing antioxidant power (FRAP) assay) of vegans was significantly lower than omnivores, despite their high fruit and vegetable intake. This was attributed to higher dietary fibre intake in vegans

which reduces the bioavailability of antioxidant molecules and polyphenols, potentially slowing recovery after exercise. Another explanation for this could be, for example, the increased lipid peroxidation seen in vegans may be a result of their excessive consumption of antioxidants, causing toxicity and pro-oxidant activity (Bouayed & Bohn, 2010). Furthermore, Nebl et al (2019) found pre-exercise markers of oxidative stress (plasma MDA and nitrate) to be elevated in vegan recreational runners compared to omnivore recreational runners while dietary antioxidant intakes were no different. This was attributed to the increased creatine intake of omnivores.

Alternatively, Dietrich et al (2022) found that markers of oxidative stress in 24 h urine (e.g., 3-nitrotyrosine (3-NT), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-iso-prostaglandin F2 α (8-iso-PGF2 α)) tended to be lower in vegans compared to omnivores. Despite these effects, further studies are needed to validate these findings as it was noted that the biomarkers of oxidative stress may have been influenced by factors such as endogenous antioxidants, and metabolic turnover rates (Dietrich et al 2022). This is similar to findings by Trapp et al (2010), albeit in vegetarians. Furthermore, a systematic review by Aleksandrova et al (2021) found in observational studies vegetarians diets exhibited lower oxidative stress biomarkers reflecting lipid peroxidation and immune-inflammatory activation as sources of oxidative stress malondialdehyde (MDA) and myeloperoxidase (MPO) as well as higher biomarkers of antioxidant defence, and ROS detoxification. Although, this was when compared to a 'Western diet' (i.e., characterised by low fruit and vegetable intake and high processed food intake).

2.10.2 Exercise-induced adaptations and performance

Overwhelmingly, research considering vegan and omnivorous diets on post-exercise adaptations and exercise performance has found no differences (Pohl et al., 2021). Hevia-Larraín et al (2021) compared a protein-matched vegan and omnivorous diet directly to discover if the source of the protein results in differing exercise-induced muscle adaptations from a 12-week, twice weekly, supervised resistance training programme in healthy young men. Protein intakes were matched using a soy protein supplement and there were minimal differences in other nutrient intakes, except a higher carbohydrate intake in the vegan group. Under these conditions, there was found to be no difference in muscle adaptations (i.e., hypertrophy (muscle cross-sectional area), fibre type (fibre cross-sectional area) and force (1 repetition maximum isotonic strength)) following resistance exercise. Furthermore, Page et al (2022) examined recreationally active vegan and omnivorous males' skeletal muscle structure and function and found no

differences despite the differences in nutritional intake. Regarding performance measures in both aerobic and anaerobic activities (e.g., VO₂ max, time to exhaustion, MIVC, isometric endurance, 1 repetition maximum strength tests, repeated sprint capacity, Wingate test), it appears that there are minimal differences in consuming a vegan or an omnivore diet (Craddock et al, 2016; Lynch et al, 2018, Vitale & Hueglin, 2021; Boutros et al, 2020).

2.10.3 Whey vs plant-based protein supplement on recovery of EIMD

The only research examining markers of EIMD in vegans vs omnivores was performed indirectly by comparing plant vs animal protein supplementation. Saracino et al. (2020) explored if providing a whey-based vs pea and rice-based protein supplement the night before damaging exercise would influence force recovery, inflammatory response, and muscle soreness. Irrespective of the source, the acute protein supplementation did not improve any measure of recovery from muscle damage. Spoelder et al (2023) compared pea vs whey protein supplementation (25 g·d⁻¹) in addition to habitual dietary protein intake (which did not differ between groups). It was found that whey protein supplementation but not pea protein attenuated CK increases at the 24 h time point following damaging exercise. This is contrary to the work by Shenoy et al (2016) who found that the long term effect of plant protein supplementation (soy) (21 g twice daily for 4 weeks) prior to damaging exercise (drop jumps) attenuated markers of EIMD and improved force recovery. One explanation for this difference could be the higher amount of supplemental protein (42 g·d⁻¹ vs 25 g·d⁻¹) used in this study as well as the use of young vs older adults (Spoelder et al, 2023).

Chapter 3 - Manuscript

3.1 Abstract

Purpose: This study investigated if the source of nutrients from vegan and omnivorous diets affects the recovery of muscle function and performance following damaging exercise.

Methods: Three (3) vegans (mean age 32.3 ± 5.77 years; 63.4 ± 16.9 kg; 173.7 ± 9.5 cm) and seven (7) omnivores (mean age 25 ± 5.35 years; 70.9 ± 8.5 kg; 167.9 ± 8.2 cm) underwent a muscle-damaging exercise protocol involving 200 drop jumps. At baseline, and 0 h, 1 h, 3 h, 24 h, 48 h, and 72 h post-exercise maximal isometric voluntary contraction (MIVC), rate of force development (RFD), countermovement jump height (CMJ), pressure pain threshold (PPT), pain at rest (overall and by specific muscle), and pain during CMJ and MIVC were measured to assess muscle function, performance and soreness. Participants followed a macronutrient-matched meal plan and consumed a standardised meal post-exercise.

Results: A significant interaction effect of time x diet on Pain Rest, and Pain MIVC existed. Post-hoc analysis found significantly lower MIVC in vegans at 24 h ($P = 0.04$), Pain Rest to be significantly higher in vegans at 3 h ($P = 0.02$), Pain during CMJ ($P = 0.015$) and MIVC ($P = 0.02$) at 24 h was significantly higher in vegans as well as Pain during MIVC at 3 h ($P = 0.02$), PPT Rectus Femoris at 1 h significantly higher in omnivores, and 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 at 3h. A significant main effect of time on MIVC, CMJ, Pain Rest (overall and by muscle), Pain CMJ and MIVC, PPT Rectus Femoris, PPT Vastus Lateralis, and PPT Vastus Medialis. No significant main effects of diet were found. Although 'large' effect sizes existed for many variables. Most nutrients involved in muscle recovery showed no differences between diets other than Vitamin C with near significance ($P = 0.054$). Based on current literature, other nutrients that may have differed but were not quantified in this study were Creatine, L-Carnitine, Vitamin D, Anthocyanins, and Ellagitannins.

Conclusion: It appeared that the omnivore diet group experienced lower reductions in MIVC as compared to the vegan diet group who experienced increased pain at rest (overall and by muscle group) and during activity. The small sample size likely prevented the findings of this study from reaching

statistical significance so further, more powerful research addressing this study's limitations should be performed before recommendations can be made.

3.2 Introduction/Background

Vegetarian, vegan and plant-based diets have existed for many years, albeit interest has surged in the 21st century, especially in the context of exercise performance. The past 5 years have seen a notable increase in academic publications (Wirnitzer, 2020). A vegan diet, characterised by exclusive consumption of plant-based foods, differs significantly from an omnivorous diet in terms of nutrient composition (North et al., 2021). Vegan diets typically provide higher carbohydrate and dietary fibre but lower fat (including lower saturated fatty acids and differing omega-3 fatty acid intakes), protein (including leucine and L-carnitine), calcium, vitamin D, vitamin B12, zinc, selenium and creatine (Clarys et al., 2014; Boutros et al., 2020; Elorinne et al., 2016; Pohl et al., 2021). Additionally, vegan diets can be rich in polyphenols due to increased consumption of fruits, vegetables, greens and beans as reflected in the Healthy Eating Index-2010 (Guenther et al., 2014).

Exercise-induced muscle damage (EIMD) is a common challenge for athletes during periods of overreaching and overtraining, causing loss of muscle force, function and performance. EIMD occurs as a result of eccentric muscular contractions and is characterised by disruptions in muscular structure, and function, delayed-onset muscle soreness (DOMS), stiffness and swelling (Byrne et al., 2004). Ultrastructural disruptions in muscle occur as sarcomeres are overstretched leading to what is described as 'popped sarcomeres' (Peake et al., 2017). This results in imbalances in calcium homeostasis, inflammation, membrane and excitation-contraction coupling dysfunction and a reduction of muscle strength (Peake et al., 2017). After these initial events follow a process of muscle fibre degeneration and subsequent regeneration (Clarkson & Sayers, 1999) which coincide with the experience of DOMS, stiffness and swelling. The extent of the loss of muscle function following muscle-damaging exercise is dependent on how trained the damaged muscle is (i.e., the repeated bout effect (Hyldahl et al., 2017)).

Nutritional factors may play a crucial role in supporting recovery after EIMD. Protein and amino acids, polyphenols, omega-3 polyunsaturated fatty acids, Vitamin D, Vitamin C and E, and creatine monohydrate have been studied for their potential impact on recovery.

Protein and amino acids may reduce markers of muscle damage, but their ability to accelerate recovery of force is inconclusive (Buckley et al., 2010; Cockburn et al., 2010; Nosaka et al., 2006; Wojcik et al., 2001; Pasiakos et al., 2014; Davies et al., 2018). Therefore, although protein and amino acid availability are important for the adaptive remodelling of skeletal muscle after exercise, there is insufficient evidence to suggest protein intake after EIMD accelerates the recovery of muscle function (Owens et al., 2018).

Polyphenols such as anthocyanins, quercetin, catechins, tart Montmorency cherries, and ellagitannins exhibit anti-inflammatory and antioxidant properties, potentially aiding in recovery from EIMD (Carey et al., 2021; McLeay et al., 2012). Despite this, most interventions (McAnulty et al., 2008; Kerksick et al., 2010; Connolly et al., 2006; Trombold et al., 2011) use supplemented forms containing large doses of the polyphenols rather than dietary sources and thus similar effects may not apply to a diet rich in polyphenols.

Omega-3 polyunsaturated fatty acids (N-3 PUFAs) (found in nuts, seeds, and oily fish) have anti-inflammatory properties (Mickleborough, 2013). Supplementation of N-3 PUFAs can reduce markers of muscle damage but fail to attenuate recovery of muscle force (DiLorenzo et al., 2014). Vitamin D, C and E have also been explored with mixed findings on their impact on muscle function recovery and DOMS reduction (Owens et al., 2018; Willis et al., 2012; Barker et al., 2013; Thompson et al., 2003; Close et al., 2005). Finally, creatine monohydrate supplementation has been shown to enhance muscle force recovery after EIMD (Cooke et al., 2009).

The nutritional discrepancies between vegan and omnivorous diets raise questions regarding their differing ability to restore muscle function after damaging exercise. Limited research has directly compared vegan and omnivorous diets' impact on muscle function recovery with most studies focusing on antioxidant status, markers of oxidative stress or if either of the diets is more favourable to support exercise-induced muscle adaptations (Hevia-Larraín et al., 2021). Some studies suggest potential disadvantages of a vegan diet such as higher oxidative stress markers and lower antioxidant status compared to omnivores (Nebl et al., 2019; Vanacore et al., 2018). Hevia-Larraín et al. (2021) compared a protein-matched vegan and omnivorous diet directly to discover if the source of protein confers an advantage/disadvantage in supporting exercise-induced muscle adaptations rather than recovery of muscle force. There was found to be no difference in muscle adaptations following resistance exercise.

Considering recovery of muscle function and markers of muscle damage Saracino et al. (2020) explored if providing a whey-based vs pea and rice-based protein supplement the night before damaging exercise would influence force recovery, inflammatory response, and muscle soreness. Irrespective of the source, protein did not improve any measure of recovery from muscle damage.

No research has looked at whether either diet supports muscle function recovery after damaging exercise. This is important as the primary goal of a competitive athlete is to perform. In a competition setting an athlete may be less concerned about maximising adaptation following damaging exercise and rather restoring muscle function to be able to perform when need be. Therefore, determining if a vegan or omnivorous diet influences an athlete's ability to do so is of value for future guidance in sports performance recommendations.

3.3 Methods

3.3.1 Participants and Recruitment

Three (3) vegans (2 males and 1 female; mean age 32.3 ± 5.77 years; 63.4 ± 16.9 kg; 173.7 ± 9.5 cm) and seven (7) omnivores (2 males and 5 females; mean age 25 ± 5.35 ; years; 70.9 ± 8.5 kg; 167.9 ± 8.2 cm) were recruited to participate in this study. This did not meet the 16 participants per group needed to ensure of statistical power and significance (see Chapter 3.3.1.1). The sampling method used was 'voluntary response sampling' which allowed participants to register their interest in partaking in this research. Social media (Instagram, Facebook), Massey University research volunteer newsletter, word-of-mouth, flyers around Massey University Albany campus, local gyms and vegan cafes/restaurants, and a prior vegan study were used to attract participants. The study population is healthy, vegan or omnivore, adults aged 18-40 years. The inclusion criteria include having followed a vegan or omnivore diet for ≥ 2 years prior to the study and having general physical fitness. The exclusion criteria include the presence of an orthopaedic condition, medical conditions (e.g., diabetes, cardiovascular disease (CVD)), receiving hormone replacement therapy, hormonal contraception, taking drugs or nutritional supplements known to be anabolic, tobacco/cigarette use, highly trained individuals (> 4 days per week). Participants were screened for eligibility verbally or via email as well as by completing a health screening questionnaire (Appendix D). Eligible participants were provided with an information sheet (Appendix E) and if interested completed an informed consent form (Appendix F). Approval for this study was granted by the Massey University Human Ethics Committee (OM1 23/06) (Appendix G).

3.3.1.1 Sample Size

The following formula was used to determine the sample size required to provide 80% statistical power at an alpha value of 5% (Margetts & Nelson, 1998):

$$N \text{ (per group)} = 2(7.9\#)/(d/SD)^2$$

= 7.9 is the factor that provides 80% (β) power and 5% (α) significance.

d = the significant difference that is meaningful to detect/effect size.

SD = standard deviation (from previous literature)

Using data from Clifford et al (2016) about Maximal Isometric Voluntary Contraction (MIVC) Recovery (considered the primary outcome of the present study) an appropriate effect size of 1 and standard deviation of 126 Nm was determined. When using the above formula this meant $n = 15.8$ (i.e., 16). Therefore, 16 participants per group are required to meet power and significance requirements.

3.3.2 Study Design and Procedures

3.3.2.1 Study design

The design of this study was a controlled, parallel study (non-randomised), in which ‘3’ vegans and ‘7’ omnivores consumed a nutrient-matched diet and were subject to muscle-damaging exercise. Changes in jump height, quadriceps MIVC and soreness were monitored during the following 72 h.

3.3.2.2 Pre-testing procedures

Prior to the trial, participants attended a familiarisation session. They were provided with a copy of the ‘Participant Information Sheet’ and completed the ‘Health Screening Questionnaire’ and ‘Consent Form’. Participants were introduced to the Sports and Exercise Science Research Laboratory at Massey University, Auckland and were taken through all of the tests/measures they were to experience on their trial days. This included soreness tests using the pain scale and algometer, muscle performance by jump height and muscle function using the strain gauge. Seat height settings were determined, as well as the dominant leg and recorded in preparation for the trial. Participants were also shown what to expect during the muscle-damaging exercise (drop jump protocol). Height was measured (using a stadiometer) and a bioelectrical impedance analysis (BIA) (InBody 230, Biospace Corp., Seoul: Korea) scan was performed

to gather data such as weight, age and lean body mass used to calculate participants' estimated energy requirements (EER).

Prior to the trial participants were provided with video and PDF instructions on how to complete a food record and were instructed to complete a 3-day food record (Appendix H) which was analysed using Foodworks (v10.0 Professional, 2019, Xyris Pty Ltd, Brisbane: Australia) and adjusted accordingly to meet the prescribed nutritional requirements (see 3.3.3.2 Matched diets). From here, participants were provided with an adjusted 'meal plan' based on their habitual intake to be consumed during the 4 days of the trial.

Participants who menstruate were asked to begin their trial during the early follicular phase (~day 3-4 from the beginning of menstruation) of their menstrual cycle to reduce any influences differing phases of the cycle may have on muscle recovery and performance (Romero-Parra et al, 2021).

3.3.2.3 Experimental Protocol

For 48 hours prior to and throughout the trial, participants were to abstain from any exercise other than the exercise protocol on day 1 of the trial. For 24 hours prior to the trial and throughout participants were to abstain from caffeine, alcohol, and nutritional and herbal supplements.

On day 1 of the trial participants arrived and underwent baseline testing. This involved measuring their muscle pain/soreness (See 3.3.4.4), taking a blood sample (See 3.3.4.3), and measuring height and BIA (InBody 230, Biospace Corp., Seoul: Korea). The participant then performed a 3-minute self-paced warm-up on a stationary bike before measuring their countermovement jump height (See 3.3.4.2) and finally muscle function via maximal isometric voluntary contraction of the quadriceps muscle (See 3.3.4.1). Following the baseline testing the participant began the muscle-damaging exercise (See 3.3.3.1). Immediately following the exercise participants repeated the same tests as the baseline above (less height and BIA) which was termed the 0 h time point. These tests were repeated 1 h and 3 h post-exercise on day 1. Between the 1 h and 3 h time points participants received a standardised meal (See 3.2.3.2) set at 10 kcal·kg bw⁻¹.

The same tests were repeated at 24 h (day 2), 48 h (day 3), and 72 h (day 4) post-exercise.

3.3.3 Interventions

3.3.3.1 Muscle-Damaging Exercise (Drop Jumps)

Participants performed a total of 200 drop jumps from a height of 0.6 m. They landed in a squat position with a ~90-degree knee joint angle and then performed a maximal effort vertical jump to a fixed height. This height was set at the height of their extended arm during a maximum vertical jump (a styrofoam ball hanging from the ceiling - adjustable height). Round one consisted of 5 sets of 20 jumps with a 2-minute rest period in between sets. Participants rested for 5 minutes before round two where they repeated the same 5 sets of 20 jumps as in round one. Every jump was separated by approximately 10 seconds.

Drop jumps have been previously demonstrated to cause significant elevations in markers of muscle damage (Howatson et al, 2012; Nosaka et al, 2006; Goodall & Howatson, 2008; Miyama & Nosaka, 2004). Typically these protocols involved 100 drop jumps but as highlighted participants in this study completed 200. This decision was based on previous Masters of Science research from Massey University's Sport and Exercise Research Laboratory that found that 100 drop jumps were insufficient to elicit muscle damage so an increased number was adopted (Woolsey, 2023; Leach, 2023).

3.3.3.2 Matched Diets

On the day of the muscle damaging protocol and the three subsequent days, until the final assessments were taken at the 72 h time point, vegan and omnivore participants both consumed an adjusted 'vegan diet' or 'omnivore diet', mimicking their specific habitual diet, to match the energy and macronutrient intake of both groups. Participants ate at their estimated energy requirement (EER) to not induce a deficit or surplus of energy. Resting metabolic rate (RMR) was determined using an appropriate predictive equation (Cunningham, 1980). A physical activity factor was applied (e.g., 1.2 for individuals sedentary most of the day). Finally, diet-induced thermogenesis (DIT) was accounted for as an assumption of 10% (Westerterp, 2004). Protein intake was set at ~1.3-1.6 g·kg⁻¹ to provide a feasible target for vegan and omnivore diets that maximise muscle protein synthesis (Morton et al, 2018). Carbohydrate and fat intakes were set within the Acceptable Macronutrient Distribution Range (AMDR) (Trumbo et al, 2002) with ~55% of energy coming from carbohydrates and the remainder of energy needs coming from fat.

Participants maintained a 4-day diet record throughout the intervention to ensure these requirements were met as per their personalised 'meal plan'. On the day of the muscle-damaging exercise, participants

consumed a separate standardised meal (e.g., a plant-based meal for the vegan group and a mixed meal for the omnivores) between the 1 h and 3 h assessment time points. This meal contained 10 kcal·kg bw⁻¹ for all participants, with each meal sharing a similar macronutrient composition.

3.3.3.3 Food Records

Participants were provided with video instructions on how to complete food records. Prior to the muscle-damaging intervention, they completed a 3-day diet record which was analysed using Foodworks (v10.0 Professional, 2019, Xyris Pty Ltd, Brisbane: Australia) and adjusted using food from their habitual diet to meet the prescribed nutritional requirements. Participants maintained a 4-day diet record throughout the intervention to ensure these requirements were met.

3.3.4 Procedures

3.3.4.1 Muscle Function

Muscle function was determined by measuring the MIVC of the quadriceps muscle of the dominant limb (measured in Newtons (N)) and rate of force development (25%, 50%, and 75%) (RFD) (measured in Newtons per second (N·s⁻¹)) during this contraction. RFD is the time from a relaxed muscle to reach ‘X’% of the maximal force upon MIVC (Maffiuletti et al, 2016). Participants performed a 3-minute stationary bike warm-up. After the warm-up they sat on a chair with a portable strain gauge attached (Chronojump 2.3.0, Boscosystem, Barcelona: Spain). The chair was adjustable to cater for each participant’s anthropometric characteristics to enable a 90-degree angle of the knee joint which was assessed with a goniometer prior to contraction. The strain gauge was attached to the ankle, immediately above the malleoli via a padded anklet. Participants were strapped at the torso, hip and leg into the chair to secure them and isolate the leg being tested (dominant limb). Participants completed three x 3-second MIVCs with 30 seconds rest between efforts. Participants received a 3-second countdown prior to each contraction. Each participant was familiarised with the test procedure and received verbal encouragement for each attempt such as to contract as “hard and fast” as possible. The mean of the three contractions was used for analysis for both MIVC and RFD using the software Chronojump 2.3.0 (Chronojump 2.3.0, Boscosystem, Barcelona: Spain).

3.3.4.2 Muscle Performance (Jump Height)

Muscle performance was determined by assessing countermovement jump performance using a jump mat (Just Jump, Probotics Inc., Huntsville: Alabama, United States of America). To perform the jumps, participants stood with feet shoulder-width apart and hands on hips. When instructed, they descended into a partial squat (until an ~ 45-degree knee angle was achieved) and jumped vertically as high as possible. Participants were to land in the same position as take-off. Participants performed three jumps with 30 seconds of standing rest in between jumps. The mean height (cm) of the three jumps was used for analysis.

3.3.4.3 Muscle Pain/Soreness

Muscle soreness was assessed as pressure pain threshold (PPT) using a handheld pressure algometer (Wagner Instruments FPN 100, Greenwich, CT, USA) as seen in similar research (Clifford et al, 2016). In a rested state (i.e., not immediately following exercise), the participant lay in a supine position and the algometer was used to apply pressure to specific, pre-marked sites. The sites assessed were the central portion (belly) of the rectus femoris, vastus lateralis, vastus medialis, and the biceps femoris (participants lay prone for this measure). This pressure was applied at a constant rate of $1 \text{ kg}\cdot\text{cm}^2\cdot\text{s}^{-1}$ until the point where the participant stated the pressure had progressed to pain/discomfort. A single measure was obtained per site during each assessment which was used for analysis.

Subjective muscle soreness was also assessed using an 11-point pain scale. Participants were shown a scale (Appendix I) and ranked their general, whole-body soreness from 0 = ‘No pain’ to 10 = ‘Excruciating’ in a rested state. This same scale was used to rank soreness of the following muscle groups in both left and right legs: gastrocnemius, gluteus maximus, hamstrings, inner thighs, outer thighs and tibialis anterior (Appendix J). Participants also used the same scale to rank their soreness in an active state during the isometric quadriceps contraction test and countermovement jump.

3.3.5 Statistical Analysis

Data was analysed using the Statistical Package for the Social Sciences (SPSS, v28 IBM Corporation, Armonk, NY). Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test for normality of data. All data were assumed normal and were expressed as mean \pm SD. Analyses were performed on absolute and relative data to eliminate the effect of baseline differences. Alpha level was set at $P < 0.05$

with differences below this level considered statistically significant. Effect sizes (η^2) were calculated with values of <0.01, 0.01-0.06, 0.06-0.14, and > 0.14 representing ‘negligible’, ‘small’, ‘medium’, and ‘large’ effect sizes respectively (Lakens, 2013).

A two-way ANOVA with repeated measures was used to examine the main effects of time and diet as well as the interaction effect between time and diet for each variable (PainR, PainCJ, PainEX, PainCalf (L & R), PainGlut (L & R), PainHam (L & R), PainIN (L & R), PainOUT(L & R), PainTib (L & R), PPTRF, PPTBF, PPTVM, PPTVL, JumpHt, MIVC, RFD25, RFD50, and RFD75). The within-subject factor is time as the same participants undergo repeated measures, and the between-subject factor is diet as each participant belongs to one of two independent groups (vegan or omnivore). Significant differences were examined using a Bonferroni post-hoc test to determine where the difference lay. Where the Greenhouse-Geisser (G-G) epsilon for main effects was below 0.75, the G-G correction was used.

As highlighted in Chapter 3.3.1, sample size requirements for statistical power and significance were not reached. Due to this, any Bonferroni post-hoc analysis performed, even when main or interaction effects were not found, was done to investigate potential trends for further studies. ANOVA P-values are reported throughout for transparency around these results and should be referred to when considering the significance of post-hoc test result.

Participant characteristics and nutritional data between diet groups were expressed as mean \pm SD. Differences were assessed using an Independent Samples t-test. In cases where the Levene’s test of equality of variances was $P < 0.05$ (and violated the assumption of equal variances), unequal variance was assumed, and Welch’s t-test was used to account for the variance heterogeneity.

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·s ⁻¹) | 1289 ± 672 | | 1123 ± 348 | | 0.702 |
| Baseline RFD 50% (N·s ⁻¹) | 859 ± 448 | | 748 ± 232 | | 0.702 |
| Baseline RFD 75% (N·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·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).

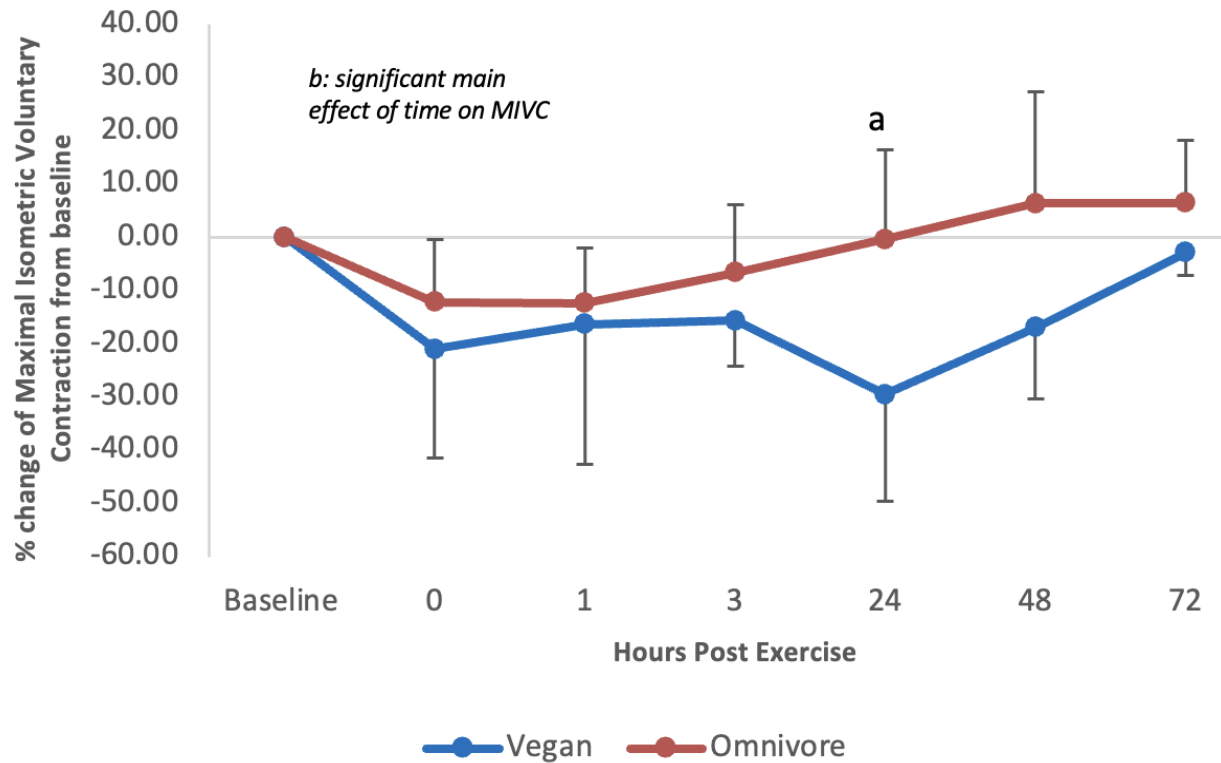


Figure 3.1. % change in MIVC over a 72 h period post muscle-damaging exercise. a: significant difference between diets ($P < 0.05$) (Bonferroni post-hoc analysis); b: significant main effect of time on MIVC ($P < 0.05$) (2-way ANOVA).

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.

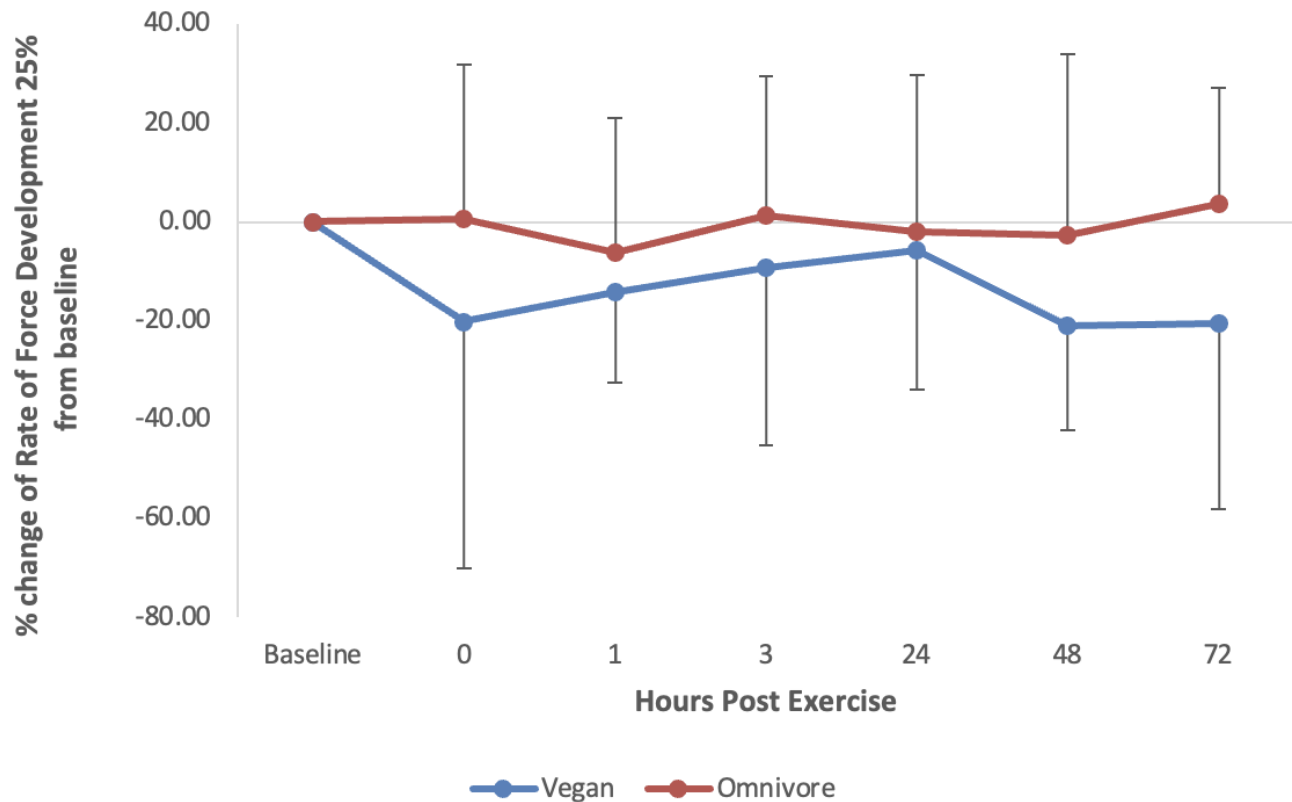


Figure 3.2. % change in RFD 25% over a 72 h period post muscle-damaging exercise.

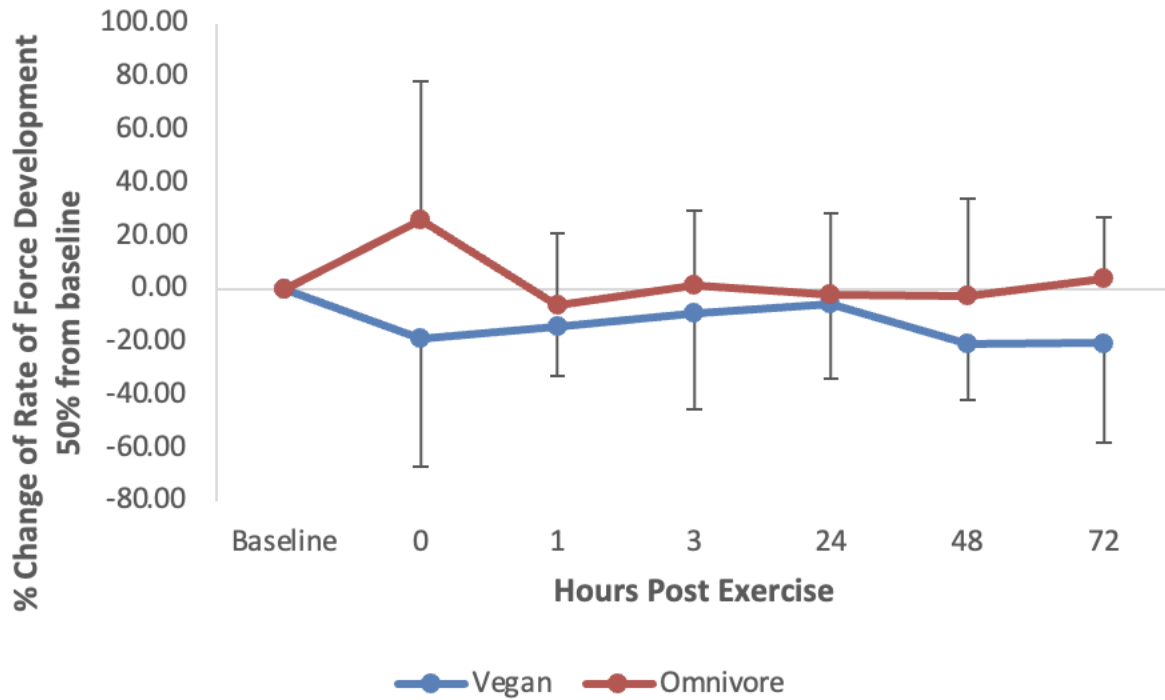


Figure 3.3. % change in RFD 50% over a 72 h period post muscle-damaging exercise.

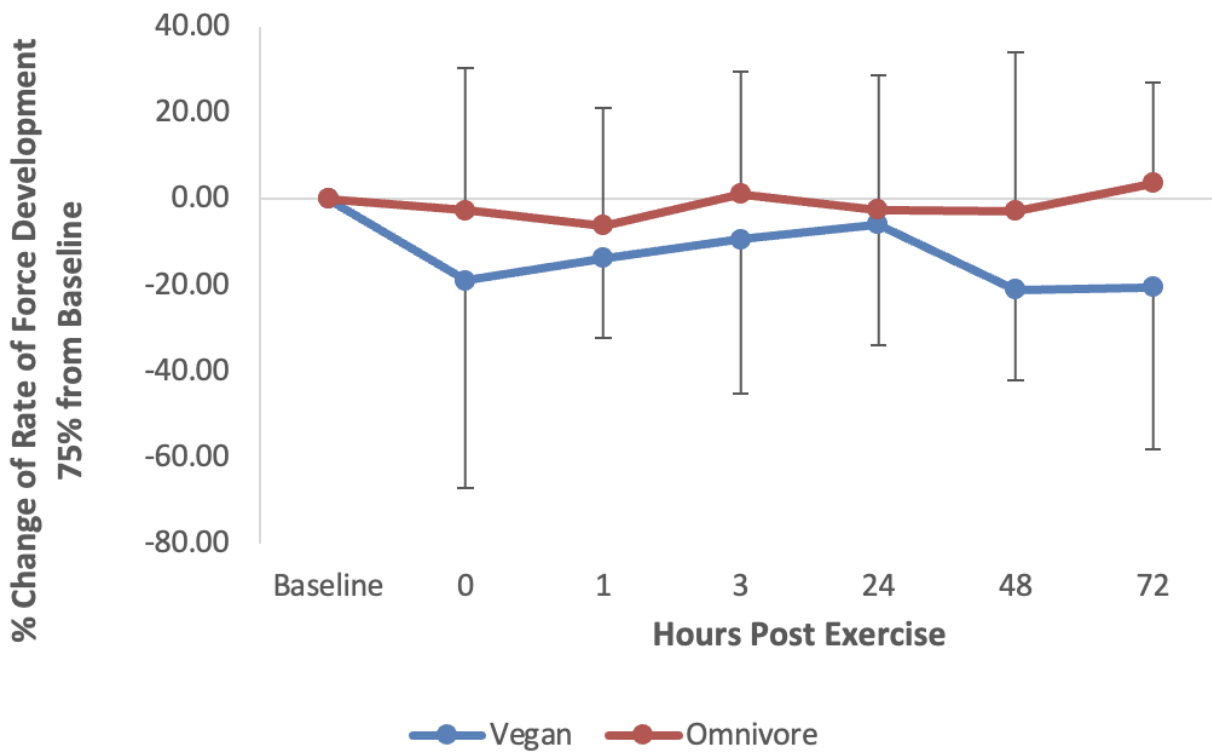


Figure 3.4. % change in RFD 75% over a 72 h period post muscle-damaging exercise.

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 largest 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 largest 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 \pm SD of 34.5 cm \pm 7.9 cm (i.e., 8.0% lower than baseline) compared to 27.2 cm \pm 14.5 cm (i.e., 23.7% lower than baseline) for Vegans, although 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).

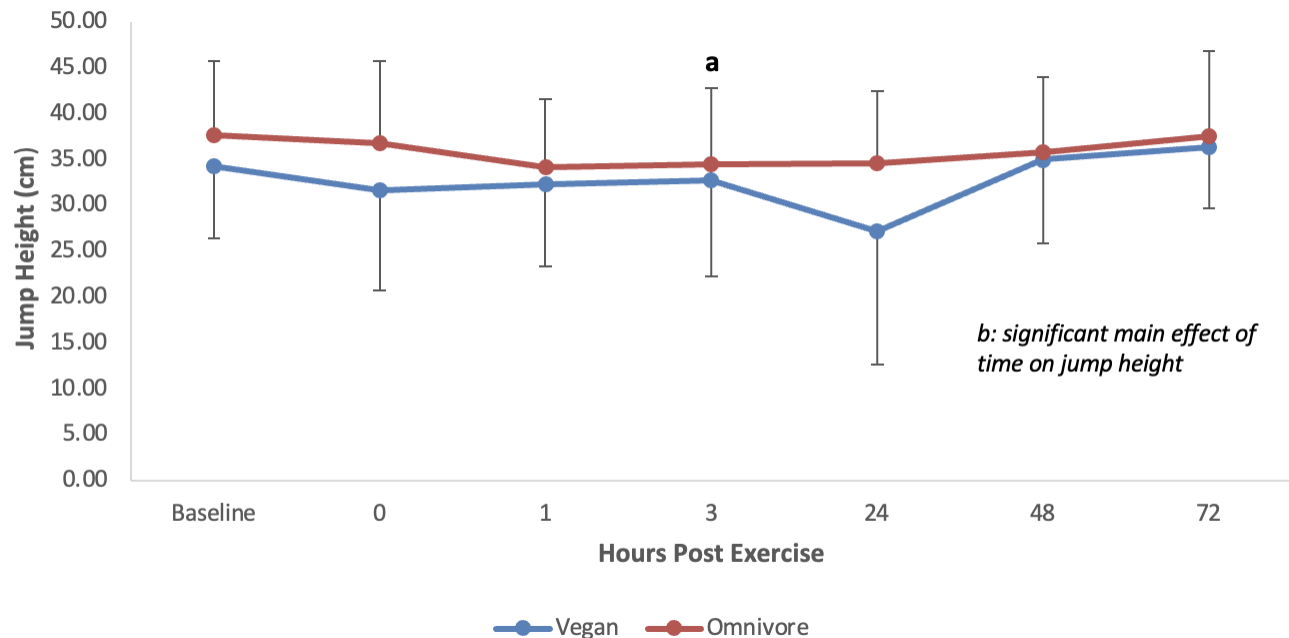


Figure 3.5. Jump Height over a 72 h period post muscle-damaging exercise. a: significant difference compared to 0 h within omnivore diet ($P < 0.05$) (Bonferroni post-hoc analysis); b: significant main effect of time on Jump Height ($P < 0.05$) (2-way ANOVA).

3.4.4 Muscle Soreness

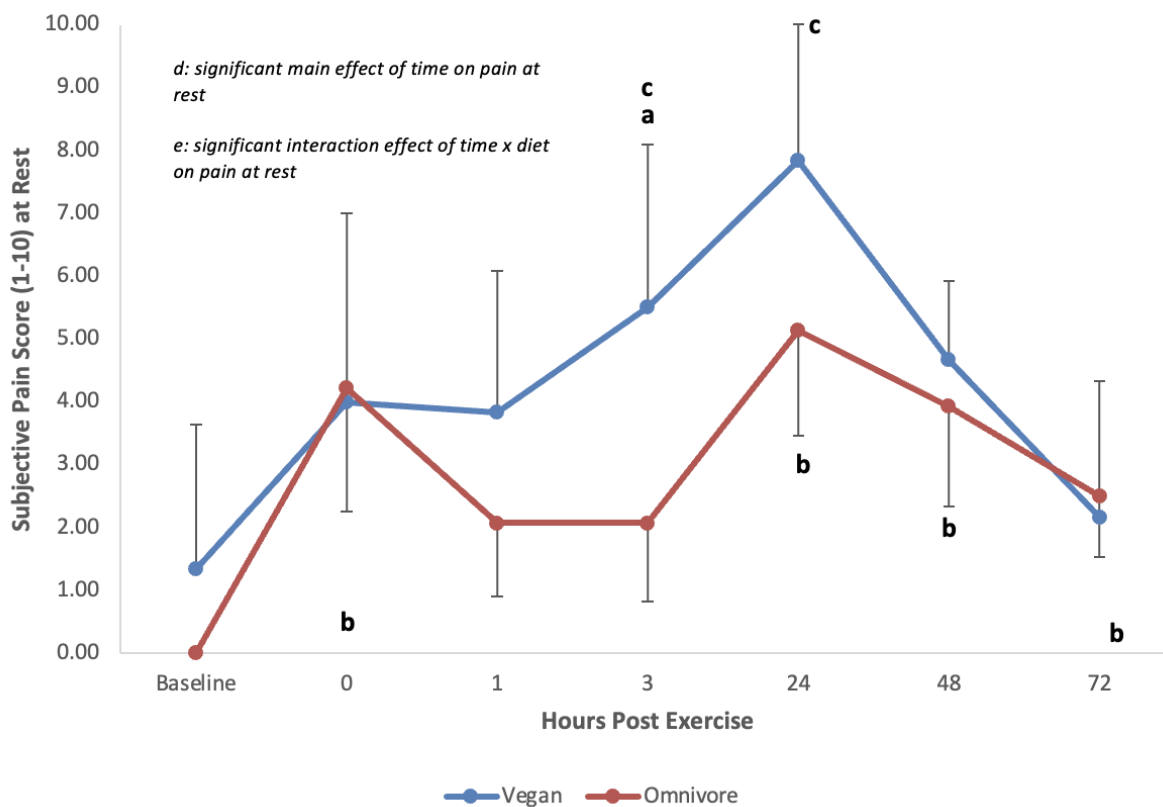
3.4.4.1 Pain Rest - General

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. Although, 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.

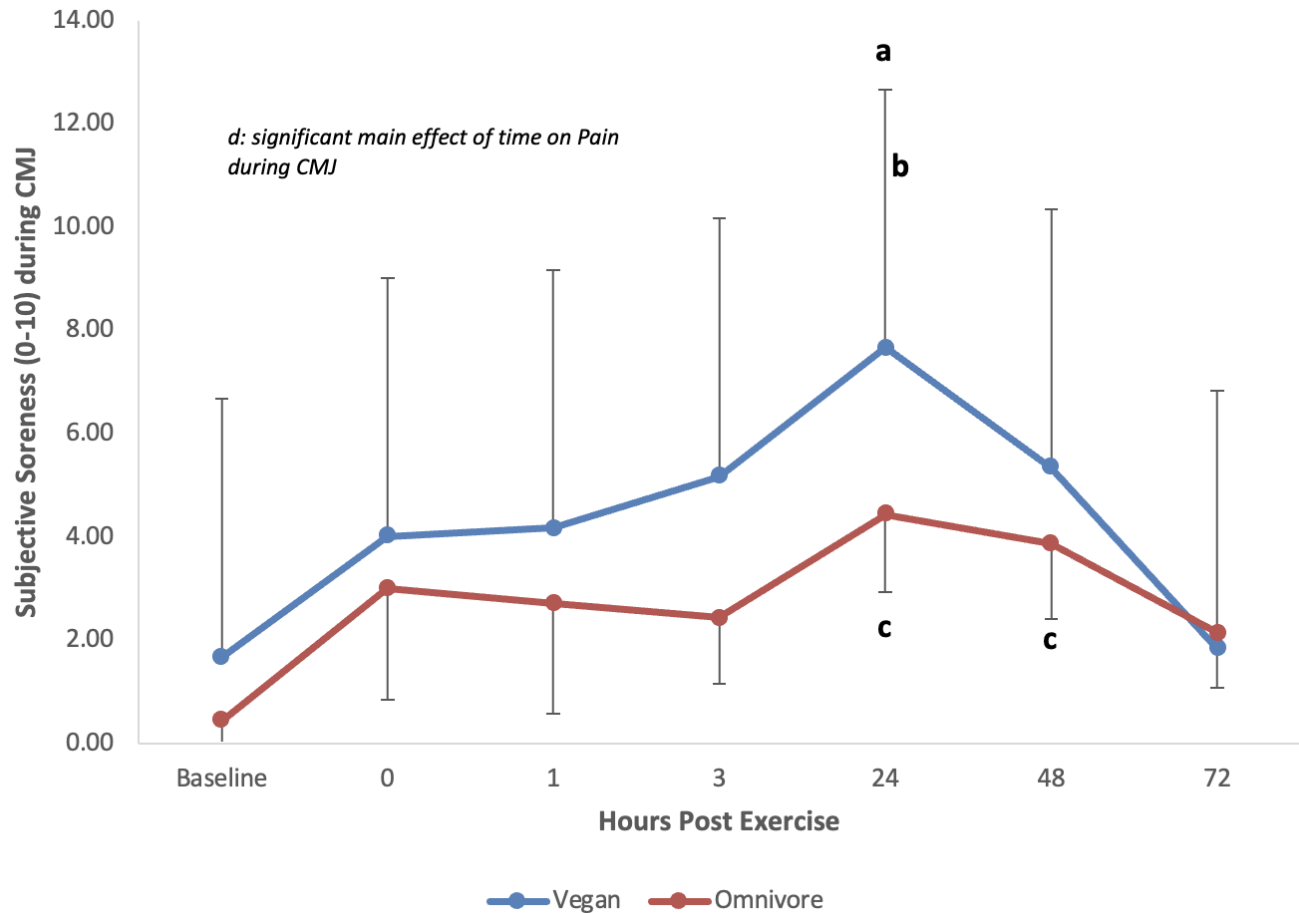


Figure 3.7. Pain during CMJ (0-10) over a 72 h period post muscle-damaging exercise. *a*: significant difference between diets ($P < 0.05$ (Bonferroni post-hoc analysis)); *b*: peak Soreness in Vegans significantly greater than baseline, 0 h, 1 h, 3 h, 72 h ($P < 0.05$) (Bonferroni post-hoc analysis); *c*: significant difference compared to baseline within omnivore 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.

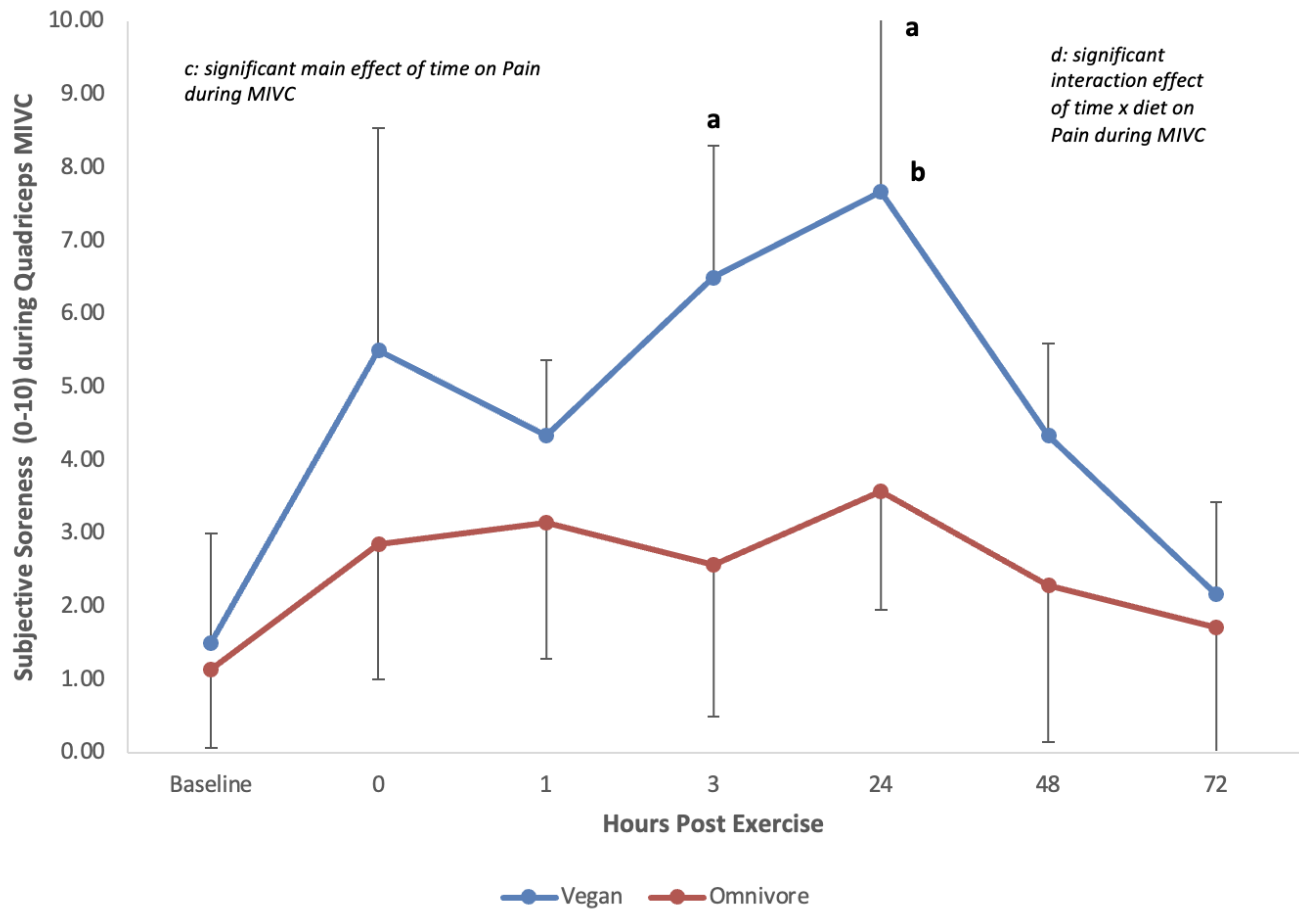


Figure 3.8. Pain during MIVC (0-10) over a 72 h period post muscle-damaging exercise. a: significant difference between diets ($P < 0.05$) (Bonferroni post-hoc analysis); b: peak Soreness in Vegans significantly greater than baseline and 72 h post exercise ($P < 0.05$) (Bonferroni post-hoc analysis); 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, $F_{1,7} = 1.452$, $P = 0.267$, $\eta^2 = 0.172$), PPT Vastus Lateralis (2-way ANOVA, $F_{1,7} = 0.790$, $P = 0.404$, $\eta^2 = 0.101$), PPT Vastus Medialis (2-way ANOVA, $F_{1,7} = 0.820$, $P = 0.395$, $\eta^2 = 0.105$), or PPT Biceps Femoris (2-way ANOVA, $F_{1,6} = 0.004$, $P = 0.951$, $\eta^2 = 0.001$). 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, $F_{2.32,16.21} = 3.477$, $P = 0.05$, $\eta^2 = 0.332$), PPT Vastus Lateralis (2-way ANOVA, $F_{3.37,23.6} = 5.384$, $P = 0.005$, $\eta^2 = 0.435$), and PPT Vastus Medialis (2-way ANOVA, $F_{2.69,18.8} = 4.680$, $P = 0.015$, $\eta^2 = 0.401$) 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, $F_{2.67,16.01} = 2.831$, $P = 0.076$, $\eta^2 = 0.321$), 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, $F_{2.32} = 1.024$, $P = 0.391$, $\eta^2 = 0.128$), PPT Vastus Lateralis (2-way ANOVA, $F_{3.37} = 0.731$, $P = 0.559$, $\eta^2 = 0.095$), PPT Vastus Medialis (2-way ANOVA, $F_{2.69} = 1.190$, $P = 0.337$, $\eta^2 = 0.145$), or PPT Biceps Femoris (2-way ANOVA, $F_{2.67} = 0.927$, $P = 0.441$, $\eta^2 = 0.134$).

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).

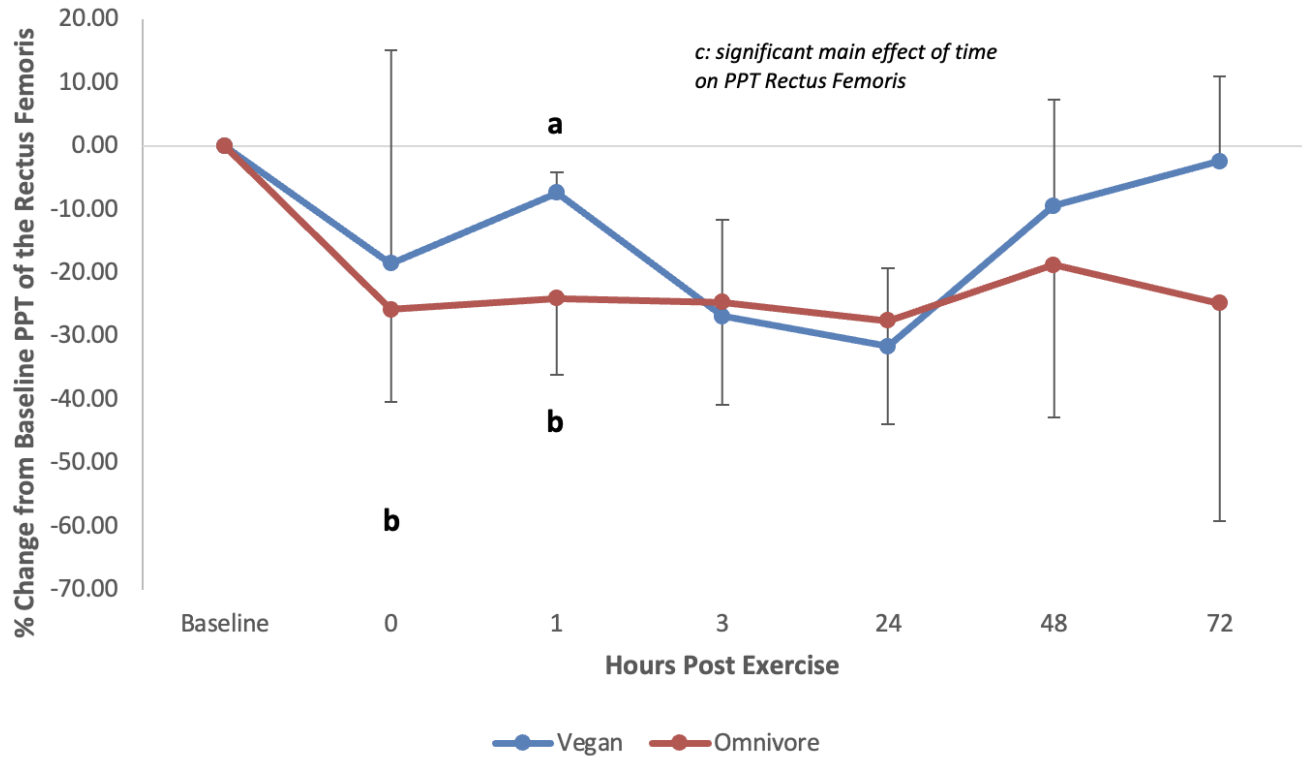


Figure 3.9. % change in PPT Rectus Femoris over a 72 h period post muscle-damaging exercise. a: significant difference between diets ($P < 0.05$) (Bonferroni post-hoc analysis); b: significant difference compared to baseline within omnivore diet ($P < 0.05$) (Bonferroni post-hoc analysis); c: significant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

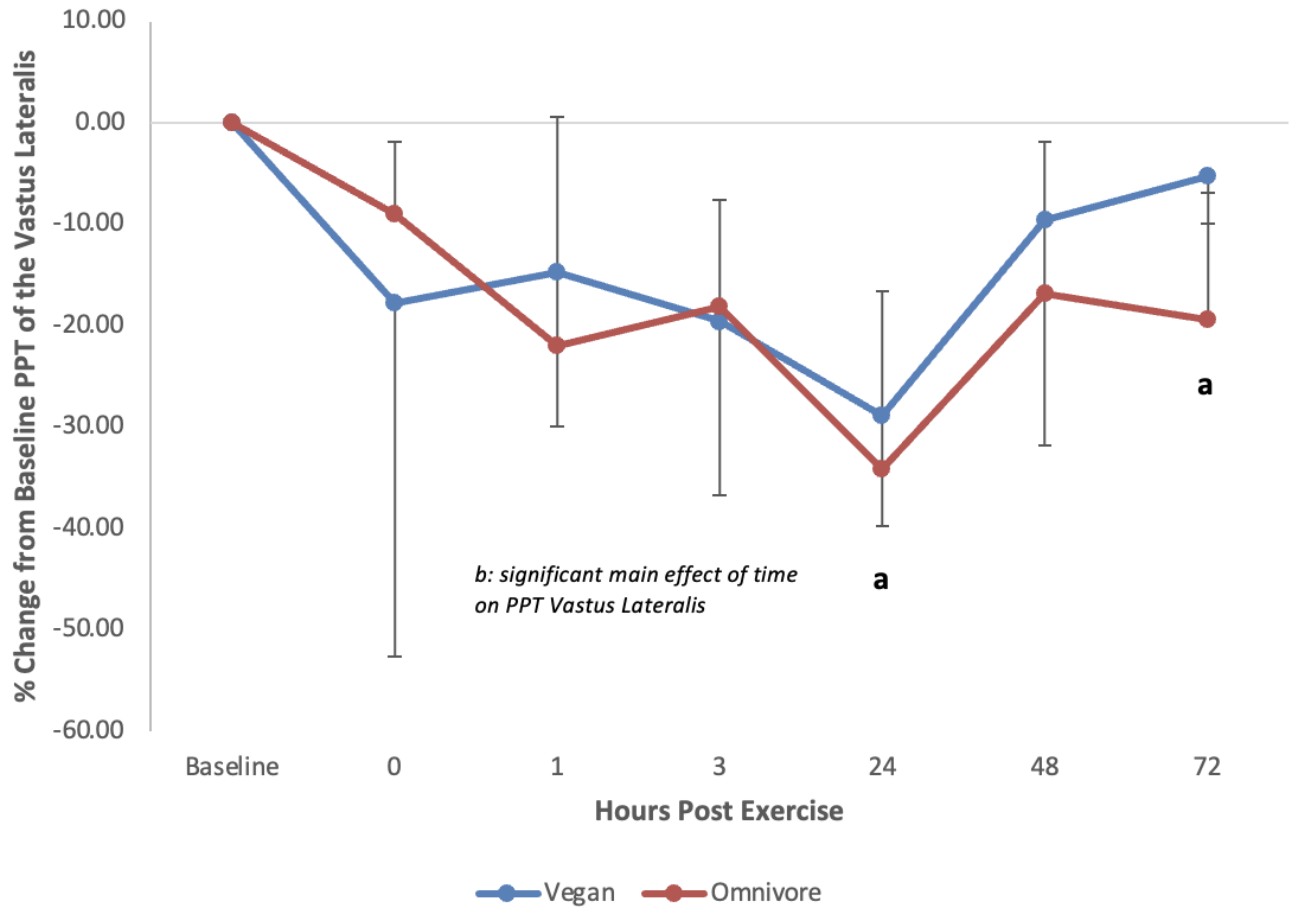


Figure 3.10. % change in PPT Vastus Lateralis over a 72 h period post muscle-damaging exercise. *a*: significant difference compared to baseline within omnivore diet ($P < 0.05$) (Bonferroni post-hoc analysis); *b*: significant main effect of time on PPT of the Vastus Lateralis ($P < 0.05$) (2-way ANOVA).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

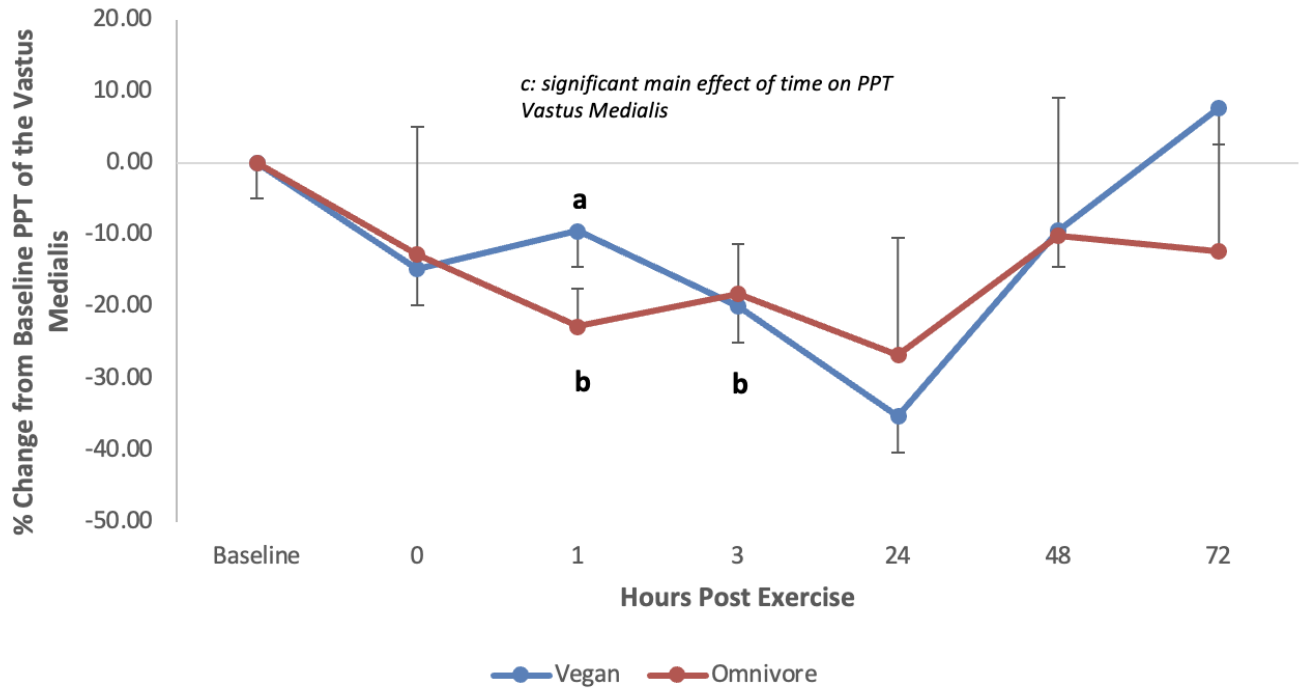


Figure 3.11. % change in PPT Vastus Medialis over a 72 h period post muscle-damaging exercise. a: significant difference between diets ($P < 0.05$) (Bonferroni post-hoc analysis); b: significant difference compared to baseline within omnivore diet ($P < 0.05$) (Bonferroni post-hoc analysis); c: significant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

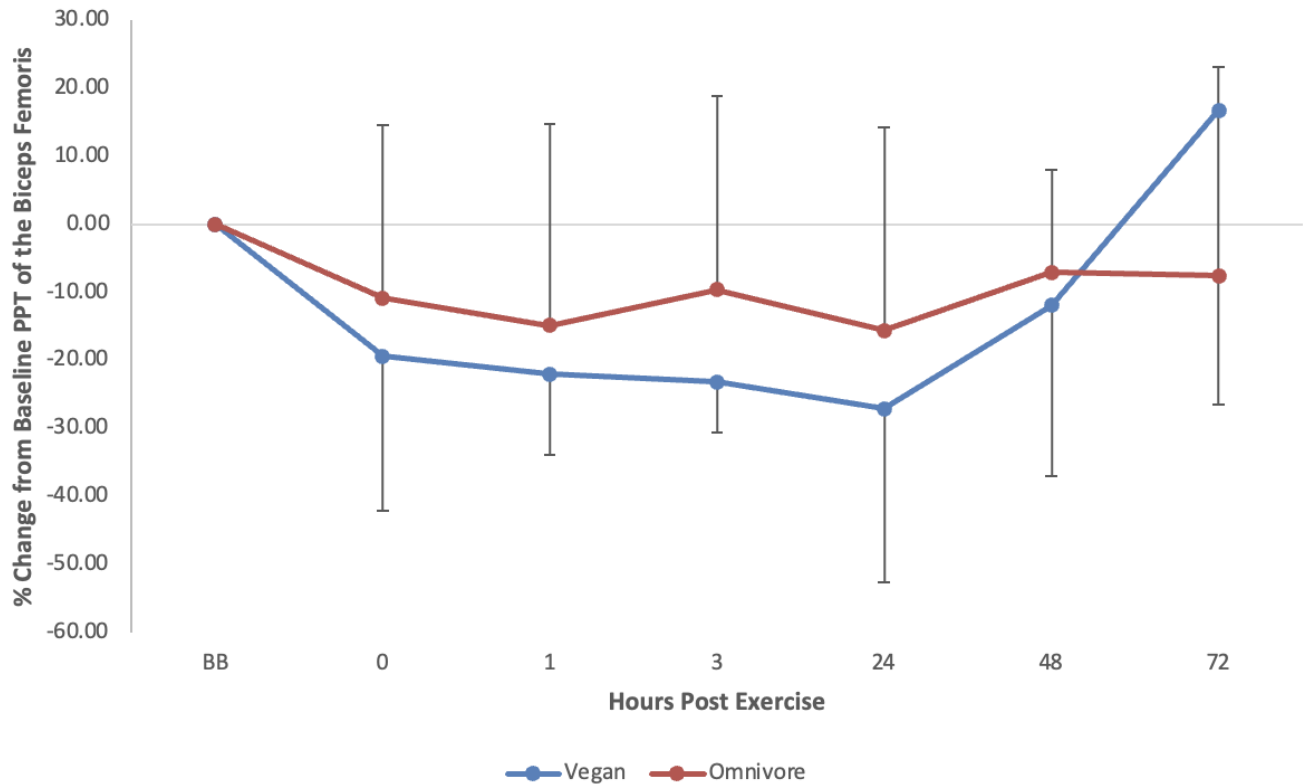


Figure 3.12. % change in PPT Biceps Femoris over a 72 h period post muscle-damaging exercise.

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.

Data table for Pain by Muscle Group in Appendix L.

3.5 Discussion

Overall the main findings of this study were that there were no significant main effects of a vegan diet or an omnivore diet on the recovery of muscle function, performance or soreness post muscle-damaging exercise. Although, large effect sizes were typically observed which suggests that with a larger sample size, the effect of diet on these variables may indeed exist, despite significance not being reached in our sample. As expected, significant main effects of time typically were present suggesting that muscle function, performance and soreness were impacted by the muscle-damaging exercise and that this exercise was sufficient to induce muscle damage in this sample. Significant interaction effects of time x diet on variables were sporadic while post-hoc analysis found some significant differences occurring between diet groups predominantly at the 1 h, 3 h and 24 h time points. Although, these significant differences found through post-hoc analysis were not accompanied by significant ANOVA effects of diet, time, or the interaction of time x diet. Initially, it appeared that participants on an omnivore diet may have experienced less of a reduction in MIVC post-damaging exercise and recovered to baseline quicker than participants on a vegan diet, although these conclusions cannot be inferred as there was no main effect of diet on recovery of MIVC. The vegan diet group were subjectively more sore following the drop jump exercise with minimal differences occurring in the PPT between groups

3.5.1 Muscle Function

3.5.1.1 Maximal Voluntary Contraction (MIVC)

Despite no significant main effect of diet or interaction effects of time x diet on MIVC being found in this sample it is important to highlight that ‘large’ effect sizes were observed. This suggests that diet may affect the recovery of MIVC as well as the change in MIVC over time between vegan and omnivore diets. This may indicate why after 24 h post damaging exercise participants consuming a vegan diet produced significantly lower force (i.e., a 29% reduction relative to baseline) than those on an omnivore diet (recovered to baseline). This 29% reduction in force is indicative of moderate EIMD whereas the most significant reduction in force experienced by omnivores (12%) is indicative of mild EIMD (Stožer et al, 2020). Perhaps if a larger sample was obtained and thus with greater statistical power, some of these findings may have been more explicit and overall interaction effects may have been significant.

When considering temporal aspects of muscle recovery there was, in fact, a significant main effect of damaging exercise impacting MIVC over time which exhibited a large effect size. Some of this initial force loss following the drop jump exercise likely occurred due to direct histological damage, whereas the majority of force loss probably was a result of the disturbance caused to E-C coupling by mechanically disrupted sarcomeres (Warren et al, 2002). Typically the greatest reduction in force after damaging exercise occurs immediately with a gradual improvement over the first 4 hours (McKune et al, 2012; Close et al, 2004; Stožer et al, 2020; Clarkson & Hubal, 2002) which was consistent with participants from both diets. However, at 24 hours force production experienced another drop which occurred to a great extent in the vegan group (i.e., being the time point of their largest reduction in force) whereas this did not occur with omnivore participants who had recovered to baseline by this time point. This second drop in force is thought to be a result of central inhibition by pain and DOMS (McKune et al, 2012; Close et al, 2004; Stožer et al, 2020; Clarkson & Hubal, 2002). Interestingly, subjective pain at rest and pain during activity (i.e., CMJ and maximal isometric contraction) peaked at the 24 h time point for vegans suggesting that this likely resulted in the second drop in force experienced by the vegan diet group. This increase in subjective pain in the vegan diet group perhaps could have been a result of BCAAs. Howatson et al (2012) demonstrated improved recovery of maximal voluntary contraction after eccentric exercise with BCAA supplementation which may be attributed to BCAAs’ ability to reduce soreness (Jackman et al, 2010). Although not quantified, there is potential that the increased soreness experienced by the vegan group could be a result of lower BCAA intake (as highlighted Rogerson, 2017)

in in this sample relative to the omnivores' BCAA intake resulting in this aforementioned central inhibition and thus force decrement at 24 h.

3.5.1.2 Rate of Force Development (RFD)

A 'medium' effect size was observed for the main effect of diet on RFD indicating the effect of diet on RFD may exist, even if it did not reach statistical significance in our sample. For example, participants on a vegan diet experienced a mean peak reduction in RFD of up to 20% whereas those on an omnivorous diet's maximal reduction was 6%. However, the high level of variability in the data due to the sample size prevented any findings from demonstrating statistical significance. Furthermore, 'small' to 'medium' effect sizes were observed for the interaction effect of time x diet on RFD.

Interestingly, no significant main effect of damaging exercise impacting RFD over time was observed, with only 'small' effect sizes observed. This is contrary to findings by Peñailillo et al (2015) which concluded that RFD (at 100-200 ms) can show greater reductions than MIVC after damaging exercise and thus is a more sensitive indirect marker of EIMD. This study found a significant main effect of damaging exercise impacting MIVC over time with a large effect size. Whereas, in this study damaging exercise did not appear to significantly impact RFD over time (notably RFD 75%, as this falls into the 100-200 ms time domain as per the findings by Peñailillo et al (2015)) and only showed a small effect size. Thus, in this case, MIVC was more sensitive to temporal aspects post-damaging exercise rather than RFD. Furthermore, research by Molina and Denadai (2012) is consistent with this study as they reported a smaller decrement and faster recovery of RFD compared with MIVC after eccentric exercise.

RFD is determined by passive mechanical properties of the muscle-tendon complex, the proportion of fast twitch muscle fibres, and output from the central nervous system (Farup et al, 2015). Eccentric exercise has been shown to induce damage in type two fibres (Cermak et al, 2012) and therefore there should be a reduction in RFD following eccentric exercise. Furthermore, central nervous system output and neural drive as measured by the rate of electromyography rise (RER) has been shown to decrease after eccentric exercise (Farup et al, 2015) which may be related to inhibition at the motor cortex level. Although, this reduction in RER may not occur until ~48-96 h post eccentric exercise and thus a linear relationship between RER and MIVC loss should not be expected. This delayed drop in RER could, in part, explain the lack of overall change in RFD observed in this study, although RFD in the vegan group

did, in fact, drop by ~20% compared to baseline at the 48 h and 72 h timepoints consistent with this concept of a delayed drop in RER.

3.5.2 Muscle Performance

3.5.2.1 Jump Height

A ‘small’ effect size was observed for the non-significant main effect of diet on jump height suggesting that it is unlikely that diet impacts jump height. Despite this, a ‘large’ effect size was seen for the interaction effect of time x diet on jump height suggesting that if a larger sample was obtained some of the findings that were not statistically significant may reach statistical significance. For example, at the 24 h time point participants on an omnivore diet jumped a mean 8% lower than baseline compared to those on a vegan diet who jumped a mean of 24% lower than at baseline. Due to baseline differences and a high amount of variability, this difference was not statistically significant, although results like this may reach significance with larger samples based on the ‘large’ effect size.

Notably, the significant main effect of damaging exercise on jump height over time observed a ‘large’ effect size suggesting that drop jumps had a substantial influence on reducing jump height.

Countermovement jump height is not necessarily impacted by MIVC (Konrad et al, 2021) but is more so determined by the ability to generate explosive force production which is essentially the RFD (Tillin et al, 2013), which RFD has been correlated with CMJ ($r = 0.68$) (McLellan et al, 2011). As discussed, RFD did not appear to be significantly impacted by the drop jump protocol but this may have been related to a delayed drop in RER. Despite RFD experiencing minimal initial reductions, CMJ was significantly reduced by the drop jump exercise over time. Although, Molina and Denadai (2012) examined the association between the isokinetic peak torque of quadriceps and peak RFD during the recovery from eccentric exercise in untrained men and found that explosive-type muscle actions seem to be less affected by muscle damage than muscular activities which involve maximum force production. This would suggest that RFD and CMJ should be less impacted by the drop jumps than MIVC.

3.5.3 Muscle Soreness

3.5.3.1 Pressure Pain Threshold

A ‘medium’ to ‘large’ effect size was observed for diet, indicating that diet may have affected PPT Rectus Femoris, PPT Vastus Lateralis and PPT Vastus Medialis. This suggests that the effect of diet on PPT for these muscles may exist, even if it did not reach statistical significance in our sample. In contrast, the effect size for diet on PPT Biceps Femoris was ‘negligible’ indicating that any effect of diet is unlikely to be of any practical significance. A similar non-significant, yet ‘medium’ to ‘large’ effect size was observed with the interaction effect of diet x time.

The main effect of damaging exercise on PPT of the muscle groups Rectus Femoris, Vastus Lateralis, and Vastus Medialis over time not only exhibited statistical significance but also displayed a ‘large’ effect size. Although not significant, the effect of damaging exercise over time on PPT Biceps Femoris was ‘large’ which suggests that the effect of damaging exercise over time on PPT Biceps Femoris may exist, even if it did not reach statistical significance in our sample. These significant effects of damaging exercise over time are consistent with the literature on DOMS suggesting that it typically peaks between 24-48 hours post-damaging exercise (Clarkson et al.,1992) and subsides by 96 hours (Cleak & Eston, 1992). For every muscle group tested, PPT experienced its greatest reduction relative to baseline at the 24 h time point (although these results did not reach significance) and for most muscle groups in each diet (except Vastus Medialis and Biceps Femoris in the Vegan diet group) had not yet returned to baseline at the 72 h time point.

For the significant differences that did occur (i.e., Vastus Medialis and Rectus Femoris PPT lower at 1 h in omnivores than vegans) these results are unexpected as DOMS (and the tenderness associated with it) is typically relatively absent in the first 8 hours after muscle-damaging exercise (Byrne et al., 2004; Nosaka & Newton, 2002). Furthermore, at the same time point, the vegan diet group reported higher subjective pain. It is likely that these findings are equivocal due to the low power of this study or the result of a type I error. Further, finding significant differences in PPT between diet groups at the 1 h time point may suggest the potential for low intra- and inter-rater reliability when collecting the data with the pressure algometer, especially as all vegan participants’ data was collected by 1 researcher alone, whereas omnivorous data had a mix of 2 researchers. Despite this, pressure algometers have been confirmed valid

and reliable (Kinser et al, 2009) especially when researchers are previously familiarised with the correct use (as they were).

Furthermore, although not measured another consequence of EIMD is swelling. Swelling can show significant increases immediately post-damaging exercise (Peake et al, 2017) and may have influenced the PPT measure if, in fact, this differed between diet groups.

3.5.3.2 Subjective Pain (Rest and Active)

Despite the lack of statistical significance found for the main effects of diet on pain at rest (overall and per specific muscle group), pain during MIVC, and pain during countermovement jump (jump height), ‘large’ effect sizes were present. This suggests that diet may have affected how sore participants get after EIMD while resting and during activity.

The significant main effect of damaging exercise on all aspects of pain over time (except tibialis anterior pain) showed a ‘large’ effect size meaning the drop jump exercise over time resulted in a substantial change in how sore participants were.

The significant interaction effect of diet x time on pain at rest, and active pain during MIVC exhibited a ‘large’ effect size hence why we found that that at the 3 h time point vegans were significantly sorer than omnivores. Furthermore, pain at rest and active pain (MIVC) peaked for both diet groups 24 h post-damaging exercise. The interaction effect of diet x time for pain at rest (by various muscle groups) and pain active (CMJ) although was not statistically significant, did experience ‘medium’ to ‘large’ effect sizes. This means that more significant differences between the two diets at various time points may exist in a future, larger sample.

Overall, it appeared that participants on a vegan diet were sorer after the exercise than those on an omnivore diet, yet in this sample, without statistical significance, this cannot be confidently concluded. There is currently minimal research evaluating pain in vegans after exercise in any form, as well as pain after injury. Although, Nadal-Nicolás et al (2021) performed a systematic review on the effect of following a vegan diet on fibromyalgia, which is characterised by chronic pain. It was concluded that following a vegan diet reduced pain at rest (contrary to the increased pain in this study), albeit it was

speculated that this occurred as a result of a reduction in body weight, leading to a lower amount of pro-inflammatory cytokines produced which would maintain pain (Zhang and An, 2007). As a result, as vegans experienced higher pain, especially in the first 24 h following the drop jump exercise, it is possible that this could be associated with a heightened initial inflammatory response. Further, it is also possible that this increased pain could simply be a feature of the limited sample (n=3) of vegans in this study.

3.5.4 Nutrients Consumed Pre and During Study

As highlighted in Chapter 2, various nutrients are efficacious in their ability to assist in the process of recovering muscle function, and of these nutrients, some are typically consumed more/less in individuals on a vegan or omnivorous diet. As presented in Appendix A, there were no significant differences in the habitual intake of all nutrients analysed other than folate. Protein intakes appeared to be much greater in the omnivorous diet (101 g·d⁻¹) vs the vegan diet (66 g·d⁻¹) yet due to the high standard deviation these differences were not statistically significant. N-3 PUFA intake appeared higher in omnivores, although yet again high variability led to non-significant differences. Vitamin C intake was much higher in omnivores (150 mg·d⁻¹) than vegans (79 mg·d⁻¹), highlighting a near-significant difference (P = 0.052).

As the participants were provided a meal plan to match their relative macronutrient and energy intakes this meant that during the trial there were no differences in protein, carbohydrate and fat intake between diet groups (Appendix B). In fact, there were no significant differences in the during-trial intake of all nutrients analysed other than niacin and Vitamin B6 which do not appear to play a key role in recovery from EIMD. However, one nutrient that was consumed in near significant (P = 0.054) greater quantities in the omnivore diet vs the vegan diet was Vitamin C. Further, the vitamin C intake of omnivores was greater than 3 times the recommended daily intake (RDI) (National Health and Medical Research Council et al, 2006) suggesting a potential ‘supplemental’ role of this high intake. Long-term (4-weeks) vitamin C supplementation (~1000 mg·d⁻¹) can delay markers of muscle recovery (e.g. CK) in non-trained, healthy men after eccentric aerobic exercise (decline treadmill running) by attenuating increases in ROS which does not appear to delay reductions in muscle function (Poulab et al, 2015; Bryer & Goldfarb, 2006). Although in our study omnivores appeared to show lower EIMD markers in the initial stages post-exercise as compared to vegans, these results were rarely statistically significant. Further, as the amount (150 mg·d⁻¹) of vitamin C consumed is only 15% of what was used in trials showing attenuation of ROS

(i.e., 1000 mg·d⁻¹), as well as omnivores actually showing evidence of improved muscle function at 24 h, it cannot be confidently suggested (and albeit, unlikely) that Vitamin C resulted in any form of ‘delayed recovery’.

Other nutrients with potential involvement in muscle recovery such as N-3 PUFAs and Vitamin E, showed no differences between groups. Despite this, the habitual intake of N-3 PUFAs (α -linolenic acid) in the vegan diet group was 1.14 g·d⁻¹ which is lower than the recommended adequate intake (AI) (National Health and Medical Research Council et al, 2006) of 1.3 g·d⁻¹ in males (Appendix B), noting the AI for females is 0.8 g·d⁻¹. As this highlights a potential deficiency in habitual intake (i.e., over the long-term) this may not result in the similar benefits in improving muscle performance seen with high-dose, acute N-3 PUFA supplementation such as found by Jakeman et al (2017). Although, Tartibian et al (2009) found a reduction in soreness 48 h after damaging eccentric exercise after longer-term (i.e., 30 days) N-3 PUFA supplementation. This was attributed to N-3 PUFA’s ability to suppress arachidonic acid, derived 2-series prostaglandins, and 4-series leukotrienes that modulate pro-inflammatory cytokines. This, in part, could explain the increased soreness experienced by the vegan diet group.

Furthermore, nutrients such as Creatine, L-carnitine, Vitamin D, Anthocyanins, and Ellagitannins were not quantified and thus may have conferred benefits for either diet group. For example, creatine and L-carnitine most likely would have been consumed in greater quantities in the omnivore diet group due to the near nil dietary sources in a plant-exclusive diet. L-carnitine’s role in the recovery after EIMD has been examined by Stefan et al (2021) who showed that 5 weeks of L-carnitine Tartrate (2 g·d⁻¹ elemental L-carnitine) improved perceived recovery and soreness, lowered serum CK, and reduced decrements in CMJ and isometric mid-thigh pull after eccentric exercise of the lower-body. Further, a review on L-carnitine intake and EIMD by Caballero-Garcia et al (2023) concluded that L-carnitine supplementation aids in recovery from muscle damage, particularly when deficient in L-carnitine. As a result, this may have conferred a benefit to omnivores, or more accurately, resulted in poorer recovery in the vegan group (due to the muscle recovery benefits of these compounds typically occurring when provided to an individual with low stores (i.e., a vegan diet)) (Fielding et al, 2018). This may partially explain why some of the findings suggested a potential improved recovery in omnivore diets. If the participants on a vegan diet were to supplement with creatine and L-carnitine this may partially level the playing field in respect to muscle recovery post EIMD.

Polyphenols such as Anthocyanins, and Ellagitannins were not quantified but as discussed in Chapter 2 a vegan diet is expected to include a higher proportion of food sources of these polyphenols (i.e., fruits, vegetables, nuts and seeds). These compounds could have played a beneficial role in recovery post-EIMD for the following various reasons. Work by McAnulty et al (2011) suggested anthocyanins' ability to increase anti-inflammatory cytokines (e.g., IL-10) which facilitates the replacement of M1 to M2 macrophages activating satellite cells involved in repair and remodelling. Koh and Tidball (2000) suggested anthocyanins increase NO by improving its bioavailability which can inhibit m-calpain activity and cytoskeletal proteolysis. Hunt et al (2021) highlighted that anthocyanins increase cellular antioxidant defence capacity through the activation of cellular redox-sensitive signalling pathways (Nrf2/ARE transcription) which results in the lowering of oxidative stress during exercise recovery.

Although, as the quantity of these compounds was not quantified it cannot be concluded if their ingestion (or lack thereof) played these roles in the muscle recovery of either diet group. Although, it is important to note that as highlighted, the literature suggests that it is feasible that they could have played a role.

Caffeine and alcohol intake was controlled and participants were instructed to not consume these substances 24 h prior to and during the trial and therefore should not have influenced the results. If caffeine was not controlled for this could have impacted results. This was demonstrated in a recent systematic review by Caldas et al (2022) who found four studies observing that caffeine ingestion between 24 h and 72 h after muscle damage attenuates the perception of pain by up to 26%. Although alcohol was controlled for, McLeay et al (2016) found that alcohol consumption following eccentric exercise does not appear to affect force recovery in females in the days following the exercise.

3.5.5 Limitations and Strengths

The main limitation of this study was the inability to reach a sufficient sample size to achieve adequate statistical power and determine statistically significant differing results between diet groups. Although trends and 'large' effect sizes occurred, these findings cannot be confidently translated into recommendations because of this limitation. Extended effort was put into the attempt to recruit sufficient participants, although many participants were lost due to ineligibility and loss of interest. Another limitation of this study was that nutritional factors such as Creatine, L-carnitine, Vitamin D,

Anthocyanins, and Ellagitannins were not quantified. These would have been valuable to measure based on their potential influence on muscle recovery.

A key strength of this study occurred with the study's design in the controlling of nutrient intake between groups as this prevented any one participant from having an acute advantage over another from an energy and macronutrient perspective during the trials. This was important as the aim of this study was to determine if when the same energy and macronutrients were consumed, does the source of these nutrients confer an advantage/disadvantage as well as help to better isolate the chronic effect of a vegan or omnivorous diet on muscle recovery? Another strength of this study was the inclusion of female participants. Currently, sporting recommendations and guidance for female athletes overly rely on male research (Emmonds et al, 2019) which may not directly apply to females. This study addressed this and further research with larger samples will be able to stratify data to determine sex-based differences.

3.5.6 Conclusion

For the most part, a lack of statistical significance plagued the results of this study with most findings being based on trends and large effect sizes. Micronutrient intake showed minimal differences between diet groups, although key nutrients involved in muscle recovery from EIMD such as Creatine, L-carnitine, Vitamin D, Anthocyanins, and Ellagitannins were not quantified. Participants on an omnivore diet appeared as if they were to experience less of a reduction in MIVC post-damaging exercise and an improved recovery to baseline than participants on a vegan diet, although statistical analysis did not support this with no main effect of diet on MIVC occurring. At some timepoints, this potentially translated to a better recovery of CMJ in the omnivore diet, although as a whole CMJ was less affected by the damaging exercise, concurrent with the minimal change in RFD. The vegan diet group appeared subjectively sorer following the drop jump exercise, although there was no main effect of diet. Minimal differences occurring in the PPT between groups.

These preliminary findings may be a result of the expected low Creatine and L-carnitine intake in vegans, high vitamin C in omnivores, low N-3 PUFAs in vegans and/or as the sample of three (3) vegans was so small there is a chance that the diets they consumed may not reflect some of the potential benefits that could be expected from a vegan diet (e.g., high polyphenol intake).

Future research should focus on recruiting a larger sample by opening the study to well-trained athletes, noting the exercise protocol may need to be adapted to ensure EIMD is induced. If needed, data can always be stratified to highly trained and non/recreationally trained participants. Further research should back up the strengths of this study by ensuring diets are macronutrient-controlled, performed in both male and female participants, and all nutrients that have potential involvement in muscle recovery should be quantified to determine what aspects of these diets confer advantages/disadvantages. This further research should also address this study's limitations before confident recommendations can be made regarding the advantages and disadvantages of either diet.

Chapter 4 - Conclusion

4.1 Summary

The overall aim of this research was to determine if the source of macronutrients (coming from either a vegan or omnivorous diet) affected the recovery of muscle function and performance after damaging exercise. We first hypothesised that participants consuming a vegan diet compared to an omnivorous diet would demonstrate an impaired recovery of knee-extensor isometric force, jump performance and muscle soreness/pain due to their expected lower intake of nutrients involved in the restoration of these variables post-damaging exercise such as n-3 PUFAs, protein, vitamin D, creatine and L-carnitine.

Although, habitually the participants in both diet groups in this study consumed similar quantities of most nutrients except for Vitamin C and protein. During the trial, macronutrients were purposely matched as per the aim of the trial to determine if the source of these macronutrients would impact recovery, hence there were no differences between diet groups here. The only nutrient with potential implications in muscle recovery which exhibited a near-significant difference between groups was Vitamin C. Also, habitual intake of N-3 PUFAs was low in the vegan diet group compared to the AI for males, which suggests a potential deficiency. Further, the intake of some nutrients were not quantified and as per the literature may have played a role in muscle recovery such as Creatine, L-carnitine, Vitamin D, Anthocyanins, and Ellagitannins.

Although macronutrients were matched and there showed no differences in many micronutrients between diet groups, the source of macronutrients did confer some differences (as well as those nutrients not quantified that likely varied between groups). This may, in part, explain how these differences resulted in the findings on recovery of muscle function, performance and soreness after EIMD.

With regard to muscle function, participants on an omnivore diet appeared to experience less of a reduction in MIVC post-damaging exercise and recovered to baseline quicker than participants on a vegan diet, although statistically no main effect of diet was found. The omnivore diet may present slight advantages in the recovery of RFD, although in this study RFD appeared less sensitive to the effects of EIMD as compared to MIVC with no significant main effect of time on RFD and only 'small' effect sizes evident.

Recovery of muscle performance (as measured via jump height) appeared as if the omnivore diet was advantageous at some time points compared to the vegan diet, although there was no main effect of diet on jump height, so inferences cannot be made into these individual time points.

Subjective soreness at rest and during activity as a result of the drop jump exercise, for the most part, appeared to impact participants on a vegan diet to a greater extent than those on an omnivorous diet. Although, objective soreness via PPT generally showed no differences except for in the initial few hours post-exercise where the vegan diet appeared to have a greater PPT. Although, as DOMS doesn't typically occur in the first 8 hours following damaging exercise this difference at the 1 h time point may be a result of low measurement intra- or inter-rater reliability, potential swelling, equivocal, or the result of a type I statistical error.

It's important to reiterate that many of these findings did not reach statistical significance and many inferences are made off the trends evident and large effect sizes which accompanied the differences that did occur. This was a result of the small sample size secondary to recruitment difficulties highlighted in the limitations in section 4.2.

These preliminary findings may be a result of the expected low Creatine and L-carnitine intake in vegans, high vitamin C in omnivores, low N-3 PUFAs in vegans and/or as the sample of three (3) vegans was so small there's a chance that the diets they consumed may not reflect some of the potential benefits that could be expected from a vegan diet (e.g., high polyphenol intake).

4.2 Limitations

This study has a large, overarching limitation that resulted in the inability to reach statistical significance with many of the findings: a small sample size. For this study to reach sufficient statistical power 16 participants per group were required (See 3.3.1.1), although as illustrated in Appendix K only 7 omnivore and 3 vegan participants completed the trial. Despite this, Appendix K highlights the extensive recruitment efforts that were made in an attempt to achieve the sample size required. Overall, 69 participants registered their interest of which 24 were vegan and 45 were omnivore. Of the 24 vegan participants, 8 attended the familiarisation sessions whereas 16 were ineligible or were unable to be

contacted. Of the 45 omnivore participants, 18 attended the familiarisation sessions whereas 27 were ineligible or were unable to be contacted. Of the 8 vegans familiarised 5 withdrew or were unable to be contacted and 3 completed the trial (37.5%). Of the 18 omnivores familiarised 11 withdrew or were unable to be contacted and 7 completed the trial (38.9%). From this, it is clear that the expected sample size was unable to be reached due to participants either being ineligible or disengaging with the study altogether/losing interest.

Concerning eligibility, hormonal contraception and training status were the main reasons potential participants became ineligible. Data (Ministry of Health, 2019) shows that 15% of New Zealand women aged 16-24, and 12% aged 35-44 use some form of long-acting reversible contraception, and 14.3% of New Zealand women dispensed combined oral contraceptives (Thomas et al, 2023) it was expected that approximately a third of female participants were likely to be ineligible.

Furthermore, initially, this study could have recruited either untrained/recreationally trained participants or highly trained participants, but not both. It was decided that a larger ‘pool’ of potential participants would likely be able to be recruited from lesser-trained individuals as there are only so many highly trained individuals. Further, the damaging-exercise protocol likely would have had to be adjusted as it was unlikely to cause significant EIMD in highly trained individuals. Also, a major deterrent for highly trained individuals is the need for them to deviate from their normal training regimen prior to and throughout the study. Although, in retrospect excluding highly trained individuals may have worked against us as a large component of this study involved rigorous exercise (200 drop jumps) which attracted trained individuals and deterred those that were less trained. Furthermore, this study would have likely been of greater interest to an athlete who is in the constant pursuit of improving their athleticism/ability and if a vegan or omnivorous diet proved advantageous this would have been of interest to them. Furthermore, although the findings of this study are useful for recreational athletes, they are more likely to inform sports nutrition guidelines and recommendations for athletes and thus would have been more transferrable findings if the research was done in athletes.

With this exclusion criterion in mind, it was paramount to advertise as much as possible to improve our likelihood of achieving the required sample size. Efforts were made to encourage potential participants highlighting that they would gain knowledge around their nutritional intake and body composition as

well as spend time with MSc students to discuss muscle recovery and nutrition. Despite the efforts, the sample size wasn't as large as intended. This was made harder as previous market research suggested the vegetarian prevalence in New Zealand to be ~20%, whereas recent data analysed from the New Zealand Health Survey 2018/19 and 2019/20 suggests the vegetarian and vegan prevalence in New Zealand is only 2.04% and 0.74% respectively (Greenwell et al, 2023).

Another limitation of this study was that there weren't as many significant differences as expected in many of the micronutrients involved in muscle recovery (e.g., N-3 PUFAs). This may have meant (and perhaps a result of the small sample size) that the vegan and omnivore diets consumed by the participants in this study may have not been a great representation of these diets in the wider population. Although participants were provided a meal plan to match their macronutrients, this was based on their habitual food intake. Further, some nutrients with potential involvement in muscle recovery were not quantified (e.g., Creatine, L-carnitine, Vitamin D, Anthocyanins, and Ellagitannins). This may have been valuable in explaining some of the findings, especially those favouring omnivores as it is expected they have a higher intake of Creatine and L-carnitine.

4.3 Strengths

A key strength of this study was that vegan and omnivorous diets were matched in the macronutrients provided to each participant (relative to their lean mass and body weight). Furthermore, participants all received a standardised meal post-exercise at the same time. This macronutrient matched diet was based on participant's habitual diets (see Chapter 3.3.3.2), with targets primarily achieved through the adjustment of portion sizes as well as introducing and/or removing extra foods. Basing these meal plans off what participants already habitually ate reduced burden for participants, in turn improving compliance. Furthermore, this controlling of nutrient intake prevented any one participant from having an advantage over another from an energy and macronutrient perspective as the aim of this study was to determine if when the same energy and macronutrients were consumed, did the source of these nutrients confer an advantage/disadvantage. This also helped to better isolate the chronic effect of a vegan or omnivorous diet on muscle recovery.

Another strength of this study was the ability to perform the research on both male and female participants. As the findings of this study may pertain importance in informing future sport nutrition

recommendations it is important to include research on females due to the current insufficiency (Emmonds et al, 2019). There are differences between the ‘female athlete’ and ‘female sporting environment’ as compared to ‘male athletes’ and as a result, findings in male research cannot always be simply applied to females. This study addressed this and if future research occurs with a larger sample, some of this data can be stratified to determine if any sex-based differences occur.

4.4 Use of findings

This is the first study to examine if when macronutrients are controlled, does the source of these macronutrients (i.e., from a vegan or omnivorous diet) influence the recovery of muscle function and performance after damaging exercise. This is important as the primary goal of a competitive athlete is to perform. In a competition setting an athlete may be less concerned about maximising adaptation following damaging exercise and rather restoring muscle function to be able to perform when need be. Furthermore, if athletes can recover faster between subsequent training sessions this would result in the ability to train more frequently at higher intensities leading to greater exercise adaptation. This is the competitive advantage athletes seek to gain. Therefore, determining if a vegan or omnivorous diet influences an athlete’s ability to recover is of value for future guidance in sports performance recommendations. Although as this study failed to reach statistical significance for many of the results one diet cannot be recommended over the other.

Instead, the findings and trends highlighted from this study should be interpreted as preliminary at most and act as a prompt for future research to explore these further while addressing the limitations of this study. Future research should focus on recruiting a larger sample by opening the study to well-trained athletes, noting the exercise protocol may need to be adapted to ensure EIMD is induced. If needed, data can always be stratified to highly trained and non/recreationally trained participants. Further research should back up the strengths of this study by ensuring diets are macronutrient-controlled and performed in both male and female participants. Finally, all nutrients that have potential involvement in muscle recovery should be quantified to determine what aspects of these diets confer advantages/disadvantages.

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Appendices

Appendix A – During Trial Nutritional Intake

| Nutrient | Nutrient Reference Values# (Recommended Daily Intake) | Omnivore (n=7) | Vegan (n=3) | T-Test (P-value*) |
|---|---|----------------|---------------|--------------------------|
| Energy (kcal) | N/A | 2319 ± 299 | 2321 ± 321 | 0.994 |
| Protein (g) | 0.84 g·kg ⁻¹ | 97.1 ± 17.7 | 89.7 ± 12.4 | 0.532 |
| Carbohydrate (g) | 45-65% of energy intake | 302 ± 37.9 | 297 ± 44.2 | 0.850 |
| Fat (g) | 20-35% of energy intake | 73.7 ± 13.5 | 77.7 ± 10.6 | 0.667 |
| N-3 PUFAs (α -linolenic acid) (g) | Adequate Intake (AI) Females: 0.8 Males: 1.3 | 1.35 ± 0.85 | 1.46 ± 0.03 | 0.829 |
| Thiamin (mg) | Females: 1.1 Males: 1.2 | 2.03 ± 1.20 | 1.94 ± 0.35 | 0.907 |
| Riboflavin (mg) | Females: 1.1 Males: 1.3 | 1.82 ± 0.80 | 2.34 ± 1.04 | 0.408 |
| Niacin Equiv (mg) | Females: 14 Males: 16 | 40.71 ± 13.06 | 26.47 ± 2.54 | 0.028[‡] |
| B6 (mg) | 1.3 | 2.17 ± 0.74 | 1.4 ± 0.06 | 0.032[‡] |
| Folate (DFE) (μ g) | 400 | 575.1 ± 185.5 | 499.7 ± 114.3 | 0.540 |
| B12 (μ g) | 2.4 | 3.86 ± 1.94 | 3.87 ± 4.01 | 0.996 |
| Vitamin C (mg) | 45 | 150 ± 77 | 79 ± 15 | 0.054 [‡] |
| Vitamin A Equiv (μ g) | Females: 700 Males: 900 | 1192 ± 1365 | 844 ± 29 | 0.680 |
| Vitamin E (mg) | Females: 7 (AI) Males: 10 (AI) | 13.4 ± 6.5 | 13.3 ± 2.6 | 0.972 |
| Sodium (mg) | N/A | 2140 ± 793 | 2930 ± 848 | 0.194 |

| | | | | |
|----------------|------------------------------------|-------------|------------|--------------------|
| Potassium (mg) | 3800 (AI) | 3550 ± 1144 | 3362 ± 380 | 0.794 |
| Magnesium (mg) | Females: 310-320 Males: 400-420 | 401 ± 134 | 416 ± 58 | 0.862 |
| Calcium (mg) | 1000 | 741 ± 366 | 869 ± 363 | 0.624 |
| Iron (mg) | Females: 18 Males: 8 | 13.3 ± 3.3 | 18.2 ± 7.5 | 0.169 |
| Zinc (mg) | Females: 8 Males: 14 | 11.9 ± 2.5 | 11.9 ± 2.6 | 0.989 |
| Selenium (µg) | Females: 60 Males: 70 | 78.3 ± 31.6 | 55.9 ± 9.7 | 0.129 [‡] |

#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.

*Significant differences between the two diet groups (P<0.05) (Independent T-test)

†Values are mean ± SD

‡Welch's T-Test used.

Appendix B – Habitual Nutritional Intake

| Nutrient | Nutrient Reference Values# (Recommended Daily Intake) | Omnivore (n=7) | Vegan (n=3) | T-Test (P-value*) |
|----------------------------------|---|----------------|-------------|--------------------|
| Energy (kcal) | N/A | 2002 ± 197 | 1736 ± 526 | 0.476 [‡] |
| Protein (g) | 0.84 g·kg ⁻¹ | 101 ± 34.1 | 66 ± 15.7 | 0.139 |
| Carbohydrate (g) | 45-65% of energy intake | 231 ± 42.9 | 201 ± 54.7 | 0.372 |
| Fat (g) | 20-35% of energy intake | 69 ± 18.8 | 68 ± 27.4 | 0.956 |
| N-3 PUFAs (α-linolenic acid) (g) | Adequate Intake (AI) Females: 0.8 Males: 1.3 | 1.83 ± 1.69 | 1.14 ± 0.58 | 0.520 |
| Thiamin (mg) | Females: 1.1 Males: 1.2 | 1.70 ± 1.31 | 1.61 ± 0.52 | 0.913 |

| | | | | |
|----------------------|------------------------------------|---------------|-------------|--------------------------|
| Riboflavin (mg) | Females: 1.1 Males: 1.3 | 1.98 ± 1.48 | 2.45 ± 2.27 | 0.699 |
| Niacin Equiv (mg) | Females: 14 Males: 16 | 41.74 ± 21.31 | 22.1 ± 9.54 | 0.174 |
| B6 (mg) | 1.3 | 2.20 ± 1.04 | 1.40 ± 0.79 | 0.272 |
| Folate (DFE) (µg) | 400 | 620.3 ± 274.6 | 347 ± 51.42 | 0.040[‡] |
| B12 (µg) | 2.4 | 4.03 ± 2.77 | 3.51 ± 4.25 | 0.820 |
| Vitamin C (mg) | 45 | 172 ± 84 | 53 ± 41 | 0.052 |
| Vitamin A Equiv (µg) | Females: 700 Males: 900 | 1299 ± 1506 | 494 ± 351 | 0.401 |
| Vitamin E (mg) | Females: 7 (AI) Males: 10 (AI) | 13.0 ± 3.0 | 13.9 ± 8.1 | 0.860 [‡] |
| Sodium (mg) | N/A | 2050 ± 819 | 2577 ± 295 | 0.323 |
| Potassium (mg) | 3800 (AI) | 3558 ± 914 | 2844 ± 1053 | 0.308 |
| Magnesium (mg) | Females: 310-320 Males: 400-420 | 373 ± 136 | 325 ± 136 | 0.623 |
| Calcium (mg) | 1000 | 749 ± 429 | 705 ± 563 | 0.895 |
| Iron (mg) | Females: 18 Males: 8 | 13.3 ± 5.6 | 13.5 ± 2.5 | 0.927 [‡] |
| Zinc (mg) | Females: 8 Males: 14 | 11.1 ± 2.9 | 9.8 ± 3.3 | 0.555 |
| Selenium (µg) | Females: 60 Males: 70 | 85.1 ± 46.4 | 40.4 ± 8.1 | 0.148 |

#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.

*Significant differences between the two diet groups (P<0.05) (Independent T-test)

†Values are mean ± SD

‡Welch's T-Test used.

Appendix C - Recruitment Poster

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 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.
- \$50 voucher to cover food and travel costs.

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 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

Appendix D – Health Screening Questionnaire



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 ¹Thomas *et al.* (1992) and ²Cardinal *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

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

1. Thomas S, Reading J and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.
2. Cardinal BJ, Esters J and Cardinal MK. Evaluation of the revised physical activity readiness questionnaire in older adults. *Med Sci Sports Exerc* 28(4): 468-472

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Appendix E – 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 Vitze. 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 and the 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:

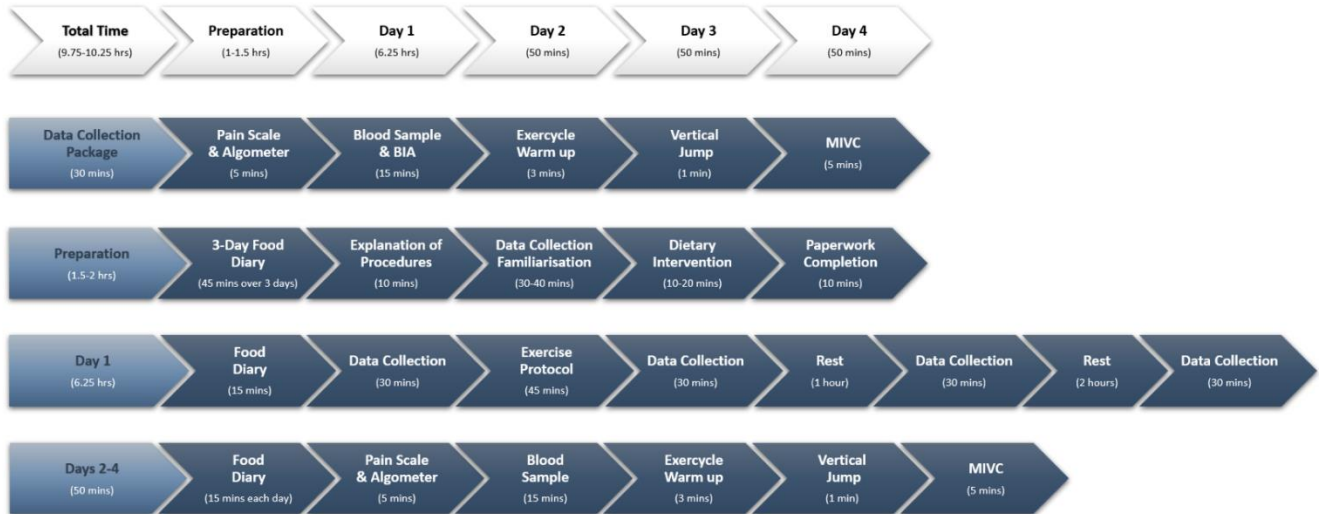
- Are advanced/elite athletes (someone who trains four or more times per week for their sport and competes at a high level)
- Are receiving hormone replacement therapy, or anabolic steroids, or oral contraceptive medication, or have a hormonal implant such as 'the rod' or an IUD (copper IUD is fine).
- 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 will return 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 from ACC 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. The results 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 Massey 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 can contact:

Dr Kaio Vitzel Senior Lecturer

School of Health Sciences sk.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.nz

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, so they can make informed decisions (tūmanako and whakapono)
- An awareness of the cultural significance and sensitivity for a culturally safe implementation of the study (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 F – Informed 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 G – Ethics Approval



Dear:

Thank you for the above application that was considered by the Massey University Human Ethics Committee:

at their meeting held on

On behalf of the Committee I am pleased to advise you that the ethics of your application are approved.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely



Dr Brian Finch Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Research Ethics Office, Research and Enterprise
Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand T 06 951 6841; 06 95106840
E humanethics@massey.ac.nz; animalethics@massey.ac.nz; gtc@massey.ac.nz

Appendix H – Food Record Template

Study ID: 103



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.

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 |

Study ID: 103

| | |
|--|--|
| | <p>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</p> |
| Dinner – Vegan lentils spaghetti Bolognese | <p>½ cup lentil sauce (see attached recipe) 1 cup spaghetti pasta (Homebrand)</p> |
| Snacks | <p>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</p> |
| Milo | <p>1 x cup Milo made with plant based Milo powder and 150mls So Good unsweetened almond milk, 100 ml hot water. No sugar</p> |

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

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

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

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

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 |

Example day

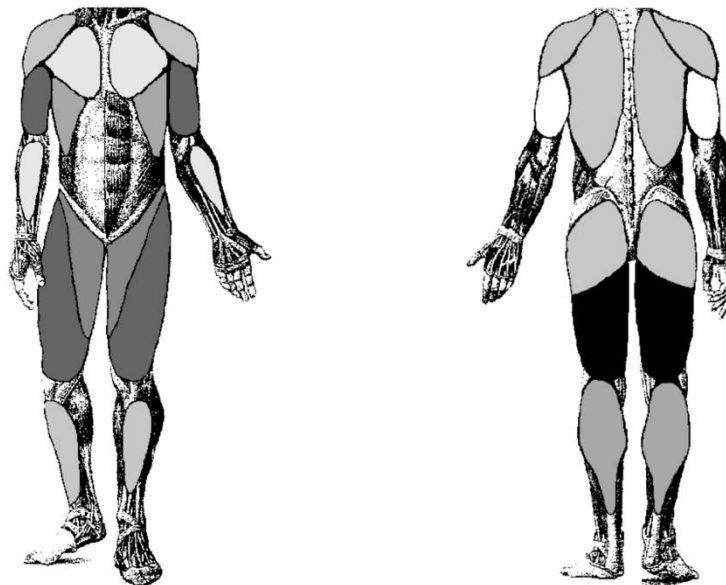
| Time food was eaten | Complete description of food (food and beverage name, brand, variety, preparation method) | Amount consumed (units, measures, weight) |
|----------------------------|---|--|
| 7:55am | Sanitarium Weetbix | 2 weetbix |
| " " | So good unsweetened almond milk | 150ml |
| " " | Chelsea 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) |
| | | |

Appendix I – Pain Scale

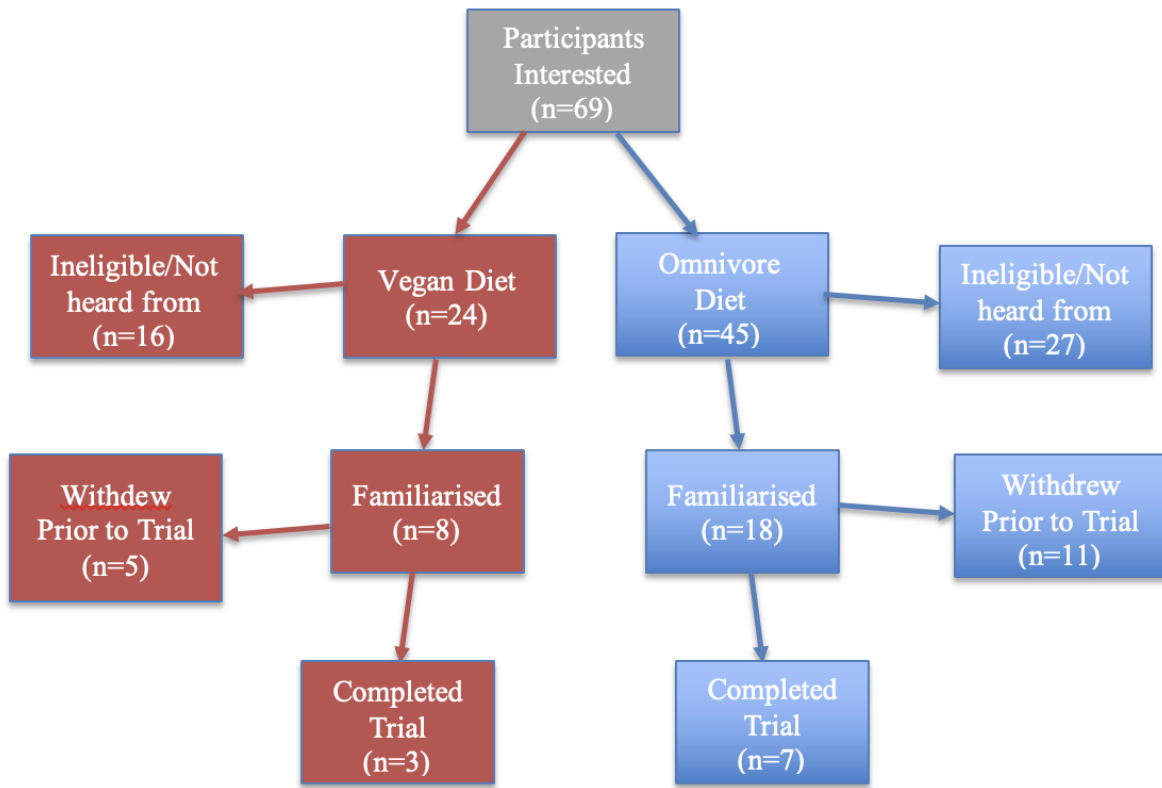
PAIN SCALE

| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|--|---|------|---|---------------|---|-------------|---|----------|---|--------------|
|  | | | | | | | | | | |
| No pain | | Mild | | Discomforting | | Distressing | | Horrible | | Excruciating |

Appendix J – Muscle Sites Diagram for Pain Scale



Appendix K – Recruitment Data



| | Vegan Diet | Omnivore Diet | Combined |
|--|------------|---------------|----------|
| % of participants familiarised that completed trial | 37.5% | 38.9% | 38.5% |
| % of total participants interested that completed trial | 12.5% | 15.6% | 14.5% |

Appendix L – Subjective Pain by Muscle Group (Mean±SD)

| <i>Mean±SD</i> | Baseline | 0 h | 1 h | 3 h | 24 h | 48 h | 72 |
|----------------|-----------------|------------|------------|------------|-------------|-------------|-----------|
| L) Calf V | 1.33±2.31 | 4.33±3.06 | 2.17±1.61 | 4.83±2.93 | 5.17±1.26 | 2.33±1.15 | 0.83±1.04 |
| L) Calf O | 0.00±0.00 | 2.86±2.33 | 1.71±1.16 | 1.64±0.44 | 4.14±1.49 | 3.00±1.41 | 1.57±1.45 |
| R) Calf V | 1.33±2.31 | 4.33±3.06 | 2.17±1.61 | 4.83±2.93 | 5.17±1.26 | 2.33±1.15 | 0.83±1.04 |
| R) Calf O | 0.00±0.00 | 2.29±2.14 | 1.71±1.25 | 1.50±0.65 | 3.86±1.07 | 2.57±0.79 | 1.21±1.07 |
| L) Glut V | 0.33±0.58 | 3.33±3.51 | 3.50±3.97 | 3.67±4.04 | 5.67±4.93 | 3.83±3.33 | 1.33±1.15 |
| L) Glut O | 0.00±0.00 | 3.14±2.49 | 1.71±1.88 | 1.36±1.25 | 4.64±2.11 | 3.64±2.30 | 1.50±1.44 |
| R) Glut V | 0.33±0.58 | 3.33±3.51 | 3.50±3.97 | 3.67±4.04 | 5.67±4.93 | 3.33±3.06 | 1.00±1.00 |
| R) Glut O | 0.00±0.00 | 3.00±2.58 | 1.71±1.98 | 1.21±1.29 | 4.93±1.24 | 3.64±2.06 | 1.50±1.44 |
| L) Ham V | 1.00±1.00 | 3.67±2.31 | 2.33±0.58 | 5.00±3.46 | 6.17±4.48 | 3.50±2.78 | 1.17±0.76 |
| L) Ham O | 0.00±0.35 | 1.57±1.20 | 1.07±1.07 | 1.64±1.35 | 3.00±1.49 | 1.93±0.93 | 0.57±0.50 |
| R) Ham V | 1.00±1.00 | 3.67±2.31 | 2.33±0.58 | 5.00±3.46 | 6.17±4.48 | 3.50±2.78 | 1.17±0.76 |
| R) Ham O | 0.00±0.00 | 1.57±1.27 | 1.07±1.10 | 1.64±1.44 | 3.00±1.41 | 1.93±0.84 | 0.57±0.53 |
| L) Inn V | 0.00±0.00 | 1.00±0.00 | 2.00±1.41 | 3.50±3.54 | 5.00±5.66 | 3.50±3.54 | 2.00±1.41 |
| L) Inn O | 0.00±0.00 | 2.14±2.19 | 2.00±1.83 | 2.14±2.17 | 3.57±3.05 | 2.50±1.76 | 1.00±0.87 |
| R) Inn V | 0.00±0.00 | 1.00±0.00 | 2.00±1.41 | 3.50±3.54 | 5.00±5.66 | 3.50±3.54 | 2.00±1.41 |
| R) Inn O | 0.00±0.00 | 2.14±2.19 | 2.00±1.83 | 2.14±2.17 | 3.57±3.05 | 2.50±1.76 | 1.07±0.98 |
| L) Out V | 0.00±0.00 | 4.00±4.24 | 2.50±2.12 | 4.00±4.24 | 3.50±0.71 | 3.50±3.54 | 2.25±1.77 |
| L) Out O | 0.07±0.19 | 3.71±2.36 | 3.50±1.89 | 3.14±2.19 | 3.79±2.88 | 3.14±1.65 | 1.43±1.13 |
| R) Out V | 0.00±0.00 | 4.00±4.24 | 2.50±2.12 | 4.00±4.24 | 3.50±0.71 | 3.50±3.54 | 2.25±1.77 |
| R) Out O | 0.07±0.19 | 3.71±2.36 | 3.07±2.32 | 3.00±2.38 | 3.79±2.88 | 3.14±1.65 | 1.57±0.98 |
| L) Tib V | 1.00±1.73 | 0.33±0.58 | 0.33±0.58 | 0.67±0.58 | 1.17±1.26 | 0.33±0.58 | 0.00±0.00 |
| L) Tib O | 0.00±0.00 | 1.00±1.46 | 1.43±1.58 | 1.00±1.13 | 0.86±0.89 | 0.64±0.50 | 0.14±0.35 |
| R) Tib V | 1.00±1.73 | 0.33±0.58 | 0.33±0.58 | 0.67±0.58 | 1.17±1.26 | 0.33±0.58 | 0.00±0.00 |
| R) Tib O | 0.00±0.00 | 1.00±1.53 | 1.43±1.62 | 1.00±1.15 | 0.86±0.90 | 0.64±0.48 | 0.14±0.38 |

L: Left, R: right, V: Vegan, O: Omnivore, Glut: Glute, Ham: Hamstring, Inn: Inner Thigh, Out: Outer Thigh, Tib: Tibialis.

Appendix M – Raw Data

| ID | Diet | PPTRFBB | PPTRF00 | PPTRF01 | PPTRF03 | PPTRF24 | PPTRF48 | PPTRF72 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1.00 | 5.50 | 4.40 | 4.40 | 4.60 | 3.50 | 6.20 | 7.50 |
| 2.00 | 1 | 3.4 | 3.3 | 2.8 | 2.9 | 2.8 | 3 | 2.4 |
| 3.00 | 1 | 7 | 4.4 | 4.9 | 4.4 | 4 | 6.8 | 4.6 |
| 4.00 | 1.00 | 3.10 | 1.80 | 1.50 | 1.40 | 2.70 | 2.10 | 1.70 |
| 5.00 | 2 | 3.3 | 2.2 | 3.1 | 2 | 2.3 | 2.7 | 3.4 |
| 6.00 | 2 | 4.5 | 2.6 | 4 | 3.1 | 2.5 | 3.6 | 3.7 |
| 7.00 | 1.00 | 7.00 | 4.40 | 4.90 | 3.80 | 6.40 | 3.10 | 2.50 |
| 8.00 | 1 | 3 | 2.7 | 3.4 | 3.7 | | 2.5 | 3 |
| 9.00 | 1 | 9 | 6.2 | 6.1 | 6.6 | 4.8 | 6.8 | 5.7 |
| 10.00 | 2 | 4 | 4.8 | 3.8 | 3.6 | 3.2 | 4.4 | 4.3 |

| ID | Diet | PPTVLBB | PPTVL00 | PPTVL01 | PPTVL03 | PPTVL24 | PPTVL48 | PPTVL72 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1.00 | 5.60 | 4.90 | 2.70 | 3.30 | 2.60 | 3.50 | 4.50 |
| 2.00 | 1 | 3.2 | 2.4 | 2.2 | 2.5 | 2.5 | 2.4 | 2.1 |
| 3.00 | 1 | 5.9 | 5 | 4 | 4.7 | 3.2 | 4.8 | 5.5 |
| 4.00 | 1.00 | 2.00 | 1.70 | 1.60 | 1.60 | 1.60 | 1.70 | 1.50 |
| 5.00 | 2 | 3.2 | 1.9 | 2.5 | 2.2 | 2 | 3.4 | 3.2 |
| 6.00 | 2 | 4 | 2.6 | 3 | 2.9 | 2.7 | 2.6 | 3.7 |
| 7.00 | 1.00 | 4.40 | 4.20 | 5.00 | 4.00 | 3.80 | 4.50 | 4.00 |
| 8.00 | 1 | 2.3 | 3 | 2.5 | 2.6 | | 2.6 | 2.2 |
| 9.00 | 1 | 7.6 | 6 | 4.5 | 5.5 | 3.8 | 4.8 | 4.8 |
| 10.00 | 2 | 3.6 | 4.4 | 3.7 | 3.6 | 3 | 3.6 | 3.3 |

| ID | Diet | PPTVMBB | PPTVM00 | PPTVM01 | PPTVM03 | PPTVM24 | PPTVM48 | PPTVM72 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1.00 | 5.70 | 4.20 | 4.20 | 4.40 | 5.00 | 6.80 | 6.00 |
| 2.00 | 1 | 2.6 | 3 | 1.7 | 1.8 | 1.4 | 1.7 | 2.2 |
| 3.00 | 1 | 5.1 | 3.7 | 3.7 | 4 | 3.8 | 4.8 | 5.5 |
| 4.00 | 1.00 | 2.20 | 1.60 | 1.70 | 1.60 | 2.00 | 2.00 | 1.60 |
| 5.00 | 2 | 3 | 2.6 | 3.2 | 2.6 | 2.3 | 3.5 | 3.2 |
| 6.00 | 2 | 4.7 | 3.1 | 3.6 | 3.2 | 2.2 | 3 | 3.8 |
| 7.00 | 1.00 | 5.70 | 4.10 | 4.60 | 5.10 | 4.50 | 4.00 | 3.20 |
| 8.00 | 1 | 2.4 | 2.7 | 2.4 | 2.6 | | 2.5 | 2.5 |
| 9.00 | 1 | 6.5 | 6 | 4.6 | 5 | 3.5 | 5.5 | 5.4 |
| 10.00 | 2 | 3.4 | 3.5 | 3 | 2.9 | 2.4 | 3.1 | 4.6 |

| ID | Diet | PPTBFBB | PPTBF00 | PPTBF01 | PPTBF03 | PPTBF24 | PPTBF48 | PPTBF72 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1.00 | 4.40 | 3.60 | 3.90 | 3.80 | 2.80 | 4.50 | 5.50 |
| 2.00 | 1 | 2.5 | 2.5 | 2.2 | 2.9 | 2.5 | 2.4 | 3.1 |
| 3.00 | 1 | 5.4 | | 4.9 | 5 | 4.6 | 5.6 | 5.6 |
| 4.00 | 1.00 | 3.60 | 1.60 | 1.70 | 1.70 | 2.20 | 2.30 | 2.50 |
| 5.00 | 2 | 3.9 | 3 | 3 | 2.8 | 2.9 | 3.5 | 3.9 |
| 6.00 | 2 | 4.5 | 2.7 | 3 | 3.3 | 2.1 | 2.8 | 3.8 |
| 7.00 | 1.00 | 5.40 | 6.00 | 6.50 | 5.70 | 7.00 | 4.60 | 3.80 |
| 8.00 | 1 | 2.8 | 3.3 | 3 | 3.4 | | 3.4 | 2.5 |
| 9.00 | 1 | 6.9 | 5.5 | 3.7 | 4.4 | 4.6 | 5.4 | 4.5 |
| 10.00 | 2 | 4.1 | 4.3 | 3.7 | 3.5 | 4 | 4.6 | 6.8 |

PPT: Pressure Pain Threshold (kg·cm²), RF: Rectus Femoris, VL: Vastus Lateralis, VM: Vastus Medialis, BF: Biceps Femoris, BB: Baseline, 00: 0 h timepoint, 01: 1 h timepoint, 03: 3 h timepoint, 24: 24 h timepoint, 48: 48 h timepoint, 72: 72 h timepoint. Diet 1: Omnivore Diet, Diet 2: Vegan Diet.

| ID | Diet | RFD25BB | RFD2500 | RFD2501 | RFD2503 | RFD2524 | RFD2548 | RFD2572 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1 | 2073.9 | 1957.87 | 1497 | 2278.63 | 2045.63 | 1885.67 | 2245.9 |
| 2.00 | 1 | 401.4 | 615.83 | 524.33 | 537.5 | 415 | 318.9 | 401.27 |
| 3.00 | 1 | 2150.73 | 2015.6 | 1653.33 | 2328.33 | 1709.93 | 2910.57 | 2378.1 |
| 4.00 | 1 | 726.4 | 391.1 | 390.07 | 345.73 | 417.43 | 322.5 | 414.37 |
| 5.00 | 2 | 761.6 | 273.03 | 514.17 | 406.2 | 514.7 | 724.73 | 900.7 |
| 6.00 | 2 | 1456.83 | 1953.83 | 1243.57 | 1818.93 | 1800.33 | 1264.4 | 1120.83 |
| 7.00 | 1 | 986.27 | 905.77 | 985.7 | 895.13 | 847.5 | 893.27 | 1047.33 |
| 8.00 | 1 | 1066 | 1338.67 | 1126.97 | 1330.55 | 1710.93 | 1651.4 | 1435.27 |
| 9.00 | 1 | 1616.2 | 1472.3 | 1898.75 | 1504.2 | 1616.07 | 1375.73 | 1769.47 |
| 10.00 | 2 | 1149.07 | 798.07 | 1197.17 | 1078.33 | 1051.53 | 633.2 | 496.47 |

| ID | Diet | RFD50BB | RFD5000 | RFD5001 | RFD5003 | RFD5024 | RFD5048 | RFD5072 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1 | 1382.6 | 1305.27 | 998 | 1519.1 | 1363.77 | 1257.13 | 1497.27 |
| 2.00 | 1 | 267.57 | 524.33 | 349.53 | 358.2 | 276.67 | 212.6 | 267.5 |
| 3.00 | 1 | 1433.8 | 1653.33 | 1102.2 | 1552.2 | 1139.97 | 1940.4 | 1585.4 |
| 4.00 | 1 | 484.3 | 260.77 | 260.03 | 230.5 | 278.3 | 214.97 | 276.23 |
| 5.00 | 2 | 507.77 | 202.23 | 342.8 | 270.8 | 343.17 | 483.17 | 600.43 |
| 6.00 | 2 | 971.23 | 1302.6 | 829.13 | 1212.63 | 1200.23 | 842.93 | 747.23 |
| 7.00 | 1 | 657.53 | 570.57 | 657.13 | 596.77 | 565 | 595.53 | 698.23 |
| 8.00 | 1 | 710.63 | 1126.97 | 751.27 | 887.05 | 1125.07 | 1100.93 | 956.83 |
| 9.00 | 1 | 1077.47 | 1898.75 | 1265.85 | 1002.8 | 1077.4 | 917.13 | 1179.67 |
| 10.00 | 2 | 766 | 532.07 | 798.1 | 718.87 | 701 | 422.13 | 330.97 |

| ID | Diet | RFD75BB | RFD7500 | RFD7501 | RFD7503 | RFD7524 | RFD7548 | RFD7572 |
|-------|------|---------|---------|---------|---------|---------|---------|---------|
| 1.00 | 1 | 691.3 | 499 | 499 | 759.53 | 681.87 | 628.53 | 748.63 |
| 2.00 | 1 | 133.8 | 205.27 | 174.77 | 179.17 | 138.33 | 106.3 | 133.73 |
| 3.00 | 1 | 716.9 | 671.87 | 551.13 | 776.13 | 570 | 970.2 | 792.7 |
| 4.00 | 1 | 242.1 | 130.33 | 130.03 | 115.27 | 139.17 | 107.5 | 138.13 |
| 5.00 | 2 | 253.87 | 101.13 | 171.4 | 135.4 | 171.6 | 241.57 | 300.23 |
| 6.00 | 2 | 485.6 | 651.3 | 423.5 | 606.3 | 600.1 | 421.43 | 373.6 |
| 7.00 | 1 | 328.73 | 301.93 | 328.6 | 298.4 | 282.5 | 297.73 | 349.13 |
| 8.00 | 1 | 355.37 | 446.2 | 375.67 | 443.5 | 562.53 | 550.47 | 478.43 |
| 9.00 | 1 | 538.73 | 490.75 | 632.95 | 501.4 | 538.67 | 458.6 | 589.8 |
| 10.00 | 2 | 383 | 266.03 | 399.07 | 359.43 | 350.53 | 211.03 | 165.5 |

RFD: Rate of Force Development ($N \cdot s^{-1}$), RFD25: Rate of Force Development 25%, RFD50: Rate of Force Development 50%, RFD75: Rate of Force Development 75%, BB: Baseline, 00: 0 h timepoint, 01: 1 h timepoint, 03: 3 h timepoint, 24: 24 h timepoint, 48: 48 h timepoint, 72: 72 h timepoint. Diet 1: Omnivore Diet, Diet 2: Vegan Diet.

| ID | Diet | MVCAvgBB | MVCAvg00 | MVCAvg01 | MVCAvg03 | MVCAvg24 | MVCAvg48 | MVCAvg72 |
|-------|------|----------|----------|----------|----------|----------|----------|----------|
| 1.00 | 1 | 476.29 | 371.77 | 383.84 | 395.68 | 425.5 | 516.28 | 559.12 |
| 2.00 | 1 | 167.45 | 186.65 | 171.12 | 190.99 | 150.94 | 139.75 | 162.92 |
| 3.00 | 1 | 308.41 | 251.12 | 240.87 | 265.06 | 252.9 | 282.65 | 304.1 |
| 4.00 | 1 | 174 | 141.49 | 130.79 | 136.28 | 171.45 | 183.57 | 158.24 |
| 5.00 | 2 | 244.01 | 135.61 | 162.63 | 181.63 | 116.5 | 166.7 | 233.3 |
| 6.00 | 2 | 251.98 | 234.1 | 249.57 | 226.56 | 214.45 | 237.89 | 257.17 |
| 7.00 | 1 | 249.65 | 200.11 | 224.64 | 227.24 | 240.81 | 244.74 | 264.09 |
| 8.00 | 1 | 230.97 | 211.08 | 227.28 | 242.51 | 306.91 | 343.38 | 284.25 |
| 9.00 | 1 | 482.86 | 439.86 | 430.96 | 464.74 | 519.38 | 526.88 | 542.57 |
| 10.00 | 2 | 409.18 | 362.79 | 349.03 | 362.35 | 321.35 | 354.15 | 383.69 |

MVC: Maximal Voluntary Contraction (Average N), BB: Baseline, 00: 0 h timepoint, 01: 1 h timepoint, 03: 3 h timepoint, 24: 24 h timepoint, 48: 48 h timepoint, 72: 72 h timepoint. Diet 1: Omnivore Diet, Diet 2: Vegan Diet.

| ID | Diet | JumpHtBB | JumpHt00 | JumpHt01 | JumpHt03 | JumpHt24 | JumpHt48 | JumpHt72 |
|-------|------|----------|----------|----------|----------|----------|----------|----------|
| 1.00 | 1 | 15.2 | 14.2 | 13.1 | 13.6 | 13.4 | 13.7 | 15 |
| 2.00 | 1 | 19.3 | 19.2 | 17.2 | 17.9 | 19.7 | 19.5 | 20.9 |
| 3.00 | 1 | 18.2 | 19.1 | 17.5 | 17.7 | 14.8 | 17.2 | 18.2 |
| 4.00 | 1 | 13.2 | 13 | 11.8 | 11.4 | 11.4 | 12 | 12.9 |
| 5.00 | 2 | 11.5 | 9 | 9.4 | 9.1 | 5.7 | 11.5 | 12.1 |
| 6.00 | 2 | 11.9 | 11.1 | 12.3 | 12.3 | 9.5 | 11.9 | 13.6 |
| 7.00 | 1 | 14.8 | 13.2 | 12.7 | 12.4 | 12.8 | 13 | 12.8 |
| 8.00 | 1 | 10.1 | 9.4 | 9.3 | 8.8 | 9.8 | 9.9 | 10.2 |
| 9.00 | 1 | 13 | 13.3 | 12.5 | 13.1 | 13.3 | 13.2 | 13.4 |
| 10.00 | 2 | 17 | 17.3 | 16.4 | 17.3 | 16.9 | 17.9 | 17.2 |

JumpHt: Countermovement Jump Height (inches), BB: Baseline, 00: 0 h timepoint, 01: 1 h timepoint, 03: 3 h timepoint, 24: 24 h timepoint, 48: 48 h timepoint, 72: 72 h timepoint. Diet 1: Omnivore Diet, Diet 2: Vegan Diet.

| ID | Diet | PainRBB | PainR00 | PainR01 | PainR03 | PainR24 | PainR48 | PainR72 |
|-------|------|----------|----------|----------|----------|----------|----------|----------|
| 1.00 | 1.00 | 0.00 | 6.00 | 2.00 | 4.00 | 8.00 | 6.00 | 2.00 |
| 2.00 | 1 | 0 | 2.5 | 0.5 | 1.5 | 4 | 4.5 | 2.5 |
| 3.00 | 1 | 0 | 6 | 4 | 2 | 7 | 4 | 4 |
| 4.00 | 1.00 | 0.00 | 2.00 | 2.00 | 2.00 | 4.00 | 1.00 | 1.00 |
| 5.00 | 2 | 0 | 4 | 6 | 7 | 9.5 | 4.5 | 2 |
| 6.00 | 2 | 4 | 7 | 4 | 7 | 9 | 6 | 3.5 |
| 7.00 | 1.00 | 0.00 | 2.00 | 1.00 | 2.00 | 4.00 | 4.00 | 3.00 |
| 8.00 | 1 | 0 | 5 | 2 | 0 | 4 | 5 | 2 |
| 9.00 | 1 | 0 | 6 | 3 | 3 | 5 | 3 | 3 |
| 10.00 | 2 | 0 | 1 | 1.5 | 2.5 | 5 | 3.5 | 1 |
| ID | Diet | PainCJBB | PainCJ00 | PainCJ01 | PainCJ03 | PainCJ24 | PainCJ48 | PainCJ72 |
| 1.00 | 1.00 | 1.00 | 6.00 | 5.00 | 4.00 | 6.00 | 6.00 | 3.00 |
| 2.00 | 1 | 0 | 3 | 2 | 2 | 4 | 4 | 2 |
| 3.00 | 1 | 0 | 5 | 6 | 2 | 7 | 5 | 2 |
| 4.00 | 1.00 | 1.00 | 1.00 | 1.00 | 3.00 | 3.00 | 2.00 | 0.00 |
| 5.00 | 2 | 2 | 6 | 7 | 8.5 | 9 | 5 | 2 |
| 6.00 | 2 | 3 | 4 | 3 | 5 | 8 | 6 | 2 |
| 7.00 | 1.00 | 0.00 | 0.00 | 2.00 | 3.00 | 4.00 | 4.00 | 3.00 |
| 8.00 | 1 | 1 | 2 | 0 | 0 | 3 | 2 | 2 |
| 9.00 | 1 | 0 | 4 | 3 | 3 | 4 | 4 | 3 |
| 10.00 | 2 | 0 | 2 | 2.5 | 2 | 6 | 5 | 1.5 |
| ID | Diet | PainEXBB | PainEX00 | PainEX01 | PainEX03 | PainEX24 | PainEX48 | PainEX72 |
| 1.00 | 1.00 | 2.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 3.00 |
| 2.00 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3.00 | 1 | 0 | 3 | 5 | 1 | 4 | 0 | 1 |
| 4.00 | 1.00 | 1.00 | 1.00 | 3.00 | 2.00 | 3.00 | 2.00 | 0.00 |
| 5.00 | 2 | 0 | 7.5 | 5.5 | 8.5 | 10 | 5.5 | 3.5 |
| 6.00 | 2 | 3 | 7 | 4 | 6 | 9 | 4.5 | 2 |
| 7.00 | 1.00 | 0.00 | 1.00 | 2.00 | 2.00 | 3.00 | 3.00 | 2.00 |
| 8.00 | 1 | 1 | 4 | 1 | 1 | 3 | 0 | 0 |
| 9.00 | 1 | 3 | 5 | 5 | 6 | 6 | 5 | 5 |
| 10.00 | 2 | 1.5 | 2 | 3.5 | 5 | 4 | 3 | 1 |

PainR: Overall Pain at Rest, PainCJ: Pain During Countermovement Jump, PainEX: Pain During MIVC, BB: Baseline, 00: 0 h timepoint, 01: 1 h timepoint, 03: 3 h timepoint, 24: 24 h timepoint, 48: 48 h timepoint, 72: 72 h timepoint. Diet 1: Omnivore Diet, Diet 2: Vegan Diet.