Vegan Diet *vs*. Omnivorous Diet: Impact on recovery of muscle function

A thesis presented in partial fulfilment of the requirements

for the degree of

Master of Science
Nutrition and Dietetics

at Massey University, Auckland, New Zealand.

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2023

ABSTRACT

Purpose: The purpose of the study was to compare the recovery of skeletal muscle function post-strenuous exercise between healthy young males on a vegan diet with healthy young males on an omnivorous diet.

Methods: Six vegan (33±6 years, 75±13 kg, 1.79±0.06 m, 23.13±2.82 kg/m^2) and six omnivore (27±7 years, 84±12 kg, 1.77±0.07 m, 27.07±4.77 kg/m^2) healthy males performed an established muscle-damaging exercise protocol, consisting of 5 sets of 20 drop jumps from a 0.6 m high platform. Skeletal muscle function was evaluated using double-legged countermovement jumps (CMJ). Generalized pain at rest and during CMJ was accessed with a visual analogue pain scale (VAS) and muscle-specific soreness at rest was assessed by asking participants to verbally rate the level of pain experienced in specific muscles of each leg. Pressure pain threshold (PPT) was used as a secondary measure of muscle-specific soreness, using a handheld algometer. These parameters were measured before drop jumps and at specific time points (0 h, 1 h, 3 h, 24 h, 48 h and 72 h following the drop jumps). 4-day food diaries were collected to access participants' habitual diets.

Results: There was no significant difference in CMJ height over time points (F=4.223, P=0.370) across the study, and diet type also had no significant effect on CMJ height (F=0.820, P=0.786). There was a significant increase for generalised pain at rest (F=10.868, P=0.002) and during CMJ (F=8.428, P=0.003), observed over time points, which was similar between diet types. Subjective pain at specific muscles at rest was also mostly similar between vegans and omnivores, except for glutes left leg (F=12.377, P=0.017) and inner thigh left leg (F=10.741, P=0.022), where vegans had less pain. PPT was not affected by the exercise protocol and was similar between vegans and omnivores, except for the calf lateral head left leg where omnivores had a slightly lower PPT (F=10.988, P=0.021).

Conclusion: The study found no differences in recovery of skeletal muscle function between omnivores and vegans, despite sporadic differences in muscle soreness. Mechanisms linking habitual diet and delayed-onset muscle soreness (DOMS) are still unclear, and the results of our study should be confirmed by larger or more powerful studies, as well as studies directed to address this specific question.

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LIST OF ABBREVIATIONS

1RM	1-repetition maximum
BCAA	Branch-chained amino acids
BMI	Body mass index
CK	Creatine kinase
CK-MM	Skeletal muscle isoform of creatine kinase
CMJ	Countermovement jump
CMJ&GPS	Countermovement jumps and generalized pain scales
CRP	C-reactive protein
DAMPs	Damage-associated molecular patterns
DJ	Drop jumps
DHA	Docosahexaenoic acid
EIMD	Exercise-induced muscle damage
EPA	Eicosapentaenoic acid
FD	Food diary
GPS	Generalized pain scales
ICC	Intraclass correlation coefficient
M1	Pro-inflammatory macrophages
M2	Anti-inflammatory macrophages
Mb	Myoglobin
MOH	Ministry of Health
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction
PA	Pressure algometer
PPT	Pressure Pain Threshold

PUFAs	Polyunsaturated fats
ROM	Range of motion
SPS	Specific pain scales
VAS	Visual analogue scales
VJ	Vertical jump
W&H	Weight and height

CHAPTER 1.
PURPOSE

1.1. Introduction

Athletes require a significant amount of training to achieve a competitive advantage in sports (Castro et al., 2011). Training or regular exercise is an essential part of any competitive sport or of a healthy lifestyle, and often incorporates elements of eccentric contractions, for example, jumping and sprinting (Byrne et al., 2004). When unaccustomed, eccentric contractions can cause exercise-induced muscle damage (EIMD) (Doma et al., 2021a). EIMD is associated with oxidative stress and inflammation, muscle soreness and reduction in skeletal muscle function (Corr et al., 2021). Several studies have indicated that EIMD can have harm the quality of training and overall performance in sports (Doma et al., 2021a).

Periods of recovery between repeated bouts of exercise are crucial for athletes to prevent a decline in subsequent performance. Optimizing recovery can enhance the training impact and minimize the breaks between the training sessions. Insufficient recovery from exercise can result in performance decline (Halson et al., 2002) or impaired training adaptation (Smith, 2000). Therefore, full recovery is vital for athletic performance (Barnett, 2006) and is important for the general population to maintain an active lifestyle. Optimal recovery between exercises maximizes fuel and hydration stores, restores skeletal muscle homeostasis, facilitates the repair of muscle fibres, and initiates muscle adaptation (Hood et al., 2006). Therefore, factors enhancing or optimizing skeletal muscle recovery are an important consideration in sports and general population training, and a range of assessment methods have been used to determine when recovery of force is optimized, i.e., have returned to baseline measurements within an expected timeframe. These methods include the measurement of skeletal muscle strength using maximal voluntary contraction (MVC) and/or 1-repetition maximum (1RM), vertical jump (VJ) or countermovement jump (CMJ), and measurement of economy/efficiency (Markus et al., 2021). Among those parameters, force is considered to be one of the most reliable methods of measuring muscle damage (Paulsen et al., 2012).

Several factors have been investigated to see whether they have an impact on muscle recovery including non-modifiable factors, such as age (Zauner et al., 1989), gender (Castro et al., 2011) and genetics (Erskine et al., 2013), and modifiable factors, including training experience (Zauner et al., 1989), amount of sleep (Castro et al., 2011), type of exercise (Tomlin and Wenger, 2001), repeated-bout effect (Peake et al., 2017) and nutrition (Pasiakos et al., 2014).

Certain aspects of the possible effect of nutrition on the recovery of skeletal muscle function have been investigated, including protein (Pasiakos et al., 2014), branch-chained amino acids

(Howatson et al., 2012), flavonoids (Carey et al., 2021), omega-3 polyunsaturated fatty acids (Smith et al., 2011), and vitamin D (Barker et al., 2013). However, recent trends in nutritional epidemiology have moved away from analysing a single element in the diet to a holistic approach analysing the impact of dietary patterns (Craddock et al., 2016). Plant-based diets are becoming increasingly popular all over the world in the general population (Leitzmann, 2014) and particularly the vegan diet, in which all sources of animal origin and derivatives are avoided, including meat, poultry, fish, eggs and dairy products. In most studies, omnivores' diets are defined as diets including habitual consumption of animal sources of protein at least once a week (Rizzo et al., 2013). The prevalence of veganism in the athletic population has not been quantified yet. There are emerging anecdotal reports that a vegan diet might provide athletic performance advantages. Vegan diets are promoted by elite athletes, including Venus Williams and Lewis Hamilton, and also promoted in popular documentaries, such as "Game Changers", further increasing its popularity (West et al., 2023).

Studies note that vegan diets are considered to be low in branch-chain amino acids, creatine, and omega-3 fatty acids due to a lack of intake of meat, eggs, fish and seafood (Vitale and Hueglin, 2021). All these nutrients have been demonstrated to be beneficial for skeletal muscle recovery. Though there are no studies that have proven that muscle recovery will be impaired when those nutrients are absent in the diet. To date, human studies investigating and comparing the impact of a vegan *vs.* an omnivore diet on skeletal muscle recovery are sparse. Moreover, due to the high diversity of dietary intake in vegan diets, there are no high-quality studies on the effect of vegan diet overall on the athletic performance (Vitale and Hueglin, 2021). Therefore, the main purpose of the study was to establish if there is a difference in muscle recovery between vegans and omnivores following a muscle-damaging exercise.

1.2. Aims, objectives and hypothesis

The research aim is to compare the recovery of skeletal muscle function post-strenuous eccentric exercise between healthy young males on a vegan diet with those on an omnivorous diet. Based on this aim, the objective of the study is to characterize the recovery of muscle function and investigate if delayed onset muscle soreness (DOMS) would contribute to any difference between the two diets.

We hypothesize there will be no significant difference in the recovery of muscle function after the muscle-damaging exercise between healthy young males on an omnivorous diet and a vegan diet.

1.3. Structure of the thesis

Chapter 2 of the thesis focuses on the mechanism and timeline of exercise-induced muscle damage and the process of skeletal muscle recovery. It starts with the importance of muscle recovery in sports, followed by the timeline of exercise-induced muscle damage, including muscle degeneration, inflammatory response, and muscle regeneration. Chapter 2 continues with the analysis of assessment methods for muscle force, skeletal muscle soreness and function. Firstly, the analysis focuses on the factors that have an impact on muscle recovery, and in particular, the impact of nutrition on muscle recovery. Secondly, the nutrient composition of the vegan diet was analysed in comparison to the omnivorous diet, followed by the analysis of specific nutrients of interest, including phytochemicals, flavonoids and polyphenols, branch-chain amino acids, creatine, and omega-3.

Chapter 3 of the thesis is presented as a research manuscript, including an abstract, introduction, methods, results, and discussion.

Chapter 4 of the thesis includes a brief overview and achievement of the aims and objectives of the study, explains of research impact, new knowledge generated and contribution to health, followed by strengths and limitations of the research and final recommendations.

1.4. Researchers' contributions

Table 1.1. Researchers' contributions

Researcher	Contribution
Associate Professor Andrew Foskett	Research design, review of ethics application, intellectual contribution to the thesis, review of thesis drafts.
Dr Kaio Vitzel	Research design, review of ethics application, intellectual contribution to the thesis, recruitment, data analysis, review of thesis drafts.
Anastasia Woolsey	Ethical application, participation in research design, recruitment, research tests, blood analysis, data collection and analysis, thesis.
Tam Leach	Ethical application, participation in research design, recruitment, research tests, data collection, and blood analysis.

CHAPTER 2.
LITERATURE REVIEW

2.1. Exercise-induced muscle damage and the process of skeletal muscle recovery: mechanism and timeline

2.1.1. Importance of optimal skeletal muscle recovery

Proper recovery is an essential part of any exercise and fitness programme (Castro et al., 2011). As an exercise bout progresses, there is a decrease in neuromuscular performance and skeletal muscle fatigue (Raeder et al., 2016). Muscle fatigue has been generally defined as "the decrease in power or force production in response to contractile activity" (Kent-Braun et al., 2012). Other definitions of muscle fatigue include loss of muscle force during the exercise (Finsterer, 2012) or inability to reach maximum force or power after the exercise (Enoka and Duchateau, 2008).

From a performance perspective, muscle recovery is defined as a return back to baseline performance measures. Another aspect of recovery is the return of metabolic fuels to pre-exercise levels, necessary for optimal muscle performance (Markus et al., 2021).

Recovery periods are necessary intervals between sets, exercises, and training sessions, and taking rest to recover is considered to be vital to ensure the quality of the next exercise bout performed (American College of Sports, 2009). Without adequate recovery, athletes may be unable to train at the required intensity/load at the next training session and may have an increased risk of injury. Furthermore, an imbalance of training and recovery periods can result in chronic fatigue and overtraining, which can have a significant impact on the athlete's health and well-being (Raeder et al., 2016). Therefore, full recovery is vital for athletic performance (Barnett, 2006) and is important for the general population to maintain an active lifestyle.

Strenuous exercise often results in EIMD, when skeletal muscles have a degree of damage that may take a few days to recover (Barnett, 2006). EIMD occurs when muscle is exposed to unaccustomed exercise, especially when exercise involves eccentric contractions (Clarkson and Hubal, 2002). Eccentric contractions occur when an external force is greater than the force produced by the muscle, resulting in muscle lengthening under tension (Howatson and van Someren, 2008). For example, when lowering submaximal weights, such as during downhill walking, when the quadriceps muscle group controls knee flexion against gravity (Proske and Morgan, 2001).

Exposure to an unaccustomed exercise causes damage to skeletal muscle, consisting of structural disruption of sarcomeres, disruption of excitation-contraction coupling and calcium signalling, resulting in the inflammatory response and activation of muscle degradation pathways (Peake et al., 2005). EIMD results in pain and reduced performance referred as

DOMS. Several studies have indicated that EIMD can impair the quality of training and overall performance in sports (Sorichter et al., 1999, Carey et al., 2021, Markus et al., 2021). Athletes require a quick return of the muscle function for the next training, and recovery strategies to minimize DOMS are widely practised (McLeay et al., 2016).

2.1.2. Process of skeletal muscle recovery: mechanism and timeline

An understanding of the process of skeletal muscle recovery is vital to the development of potential strategies to enhance it. After the skeletal muscle is injured, it goes through a process of muscle degeneration, inflammation, regeneration, and remodelling and repair (Urso, 2013). The post-exercise inflammatory response appears to be vital to further processes of muscle recovery and regeneration (Millet et al., 2011).

2.1.2.1. Skeletal muscle degeneration

The process of skeletal muscle degeneration starts with exercise-induced damage, causing damage to the structure of the muscle fibre, extracellular matrix and sarcomeres and t-tubules of the muscle tissue. Sarcomeres have different lengths and respond differently to the lengthening of the muscle through the eccentric contraction, where some weaker sarcomeres can be stretched beyond the overlap of actin and myosin fibres (Sorichter et al., 1999). This results in structural signs of muscle damage: damaged and overextended sarcomeres, Z-line streaming, myofilaments and t-tubule damage (Proske and Morgan, 2001). This mechanical damage is associated with the loss of strength straight after strenuous exercise. Muscle damage also impacts other muscle tissue structures, including costameres, intermediate filament proteins and extracellular matrix, playing an important part in the lateral transmission of force, which contributes to the decrease of strength observed after strenuous exercise (Baumert et al., 2016).

This structural damage is associated with an increased permeability of the sarcolemma. It is followed by an extracellular Ca^{2+} influx into the skeletal muscle fibre. Accumulation of excessive intracellular Ca^{2+} activates Ca^{2+}-sensitive proteases (calpains). Calpain activation leads to proteolysis of cytoskeletal and costameric proteins (Thiebaud, 2012). Increased permeability of the sarcolemma is associated with elevated levels of plasma creatine kinase (CK) and myoglobin (Baird et al., 2012). This results in distorted excitation-contraction coupling leading to further muscle damage and stiffness post-exercise (Gissel and Clausen, 2001), which seems to be another contributing factor to strength loss after strenuous exercise. This intra- and extracellular damage results in the accumulation of the damaged proteins requiring removal, and triggers inflammatory response and further proteolysis through the activation of the ubiquitin-proteasome pathway (Tidball, 2005).

All these processes jointly are associated with reduced skeletal muscle strength and a significant decrease in force production after exercise (Hyldahl et al., 2015, MacIntyre et al., 1996). Different patterns of strength return to baseline measures have been identified, with some studies reporting 24 hours (Newham et al., 1983), and some reporting 3 to 6 days (Fridén et al., 1983).

2.1.2.2. Inflammatory response

The next step in the process of skeletal muscle recovery is the inflammatory response. At the early stage of injury, neutrophils are rapidly recruited to the inflammation site by damage-associated molecular patterns (DAMPs) (Kovtun et al., 2018). Neutrophils migrate to the injury site, where they clear damaged muscle tissue through phagocytosis and by releasing proteolytic enzymes and reactive oxygen species (ROS) (Peake et al., 2017). Neutrophils dominate at the early stages of inflammation and set up the base for the repair of skeletal muscle tissue, created by macrophages. Macrophages replace neutrophils within 24 hours and can still be present for up to 14 days after the exercise (Malm et al., 2000). Between 4 and 24 hours after the muscle-damaging exercise pro-inflammatory macrophages (M1) infiltrate the muscle, phagocytosing damaged tissues, assisting the removal of cellular debris (Peake et al., 2005) and secreting pro-inflammatory cytokines, characterised by the production of IL-1β and TNF-α (Dinarello, 2000).

Pro-inflammatory cytokines activate satellite cells differentiation and stimulate the production of IL-6 and IL-10 by other leucocytes, which will work on the transition from the pro-inflammatory stage to the anti-inflammatory stage, to reduce inflammation (Peake et al., 2017). After 24 hours, the pro-inflammatory macrophages population reaches its peak and is replaced by anti-inflammatory macrophages (M2), secreting anti-inflammatory cytokines (IL-10, IL-4 and IL-13), and $CD8^+$ and T-regulatory lymphocytes, which rescue satellite cells from apoptosis and promotes their activation (Baumert et al., 2016).

Inflammation signalling molecules promote vasodilation and increase vascular permeability which causes oedema, which compresses nerve terminals in skeletal muscle and creates muscle swelling, associated with pain sensation, which appears within 24 hours after the swelling, peaking between 48 and 72 hours, and can still be present 3-4 days after the exercise (Proske and Allen, 2005).

2.1.2.3. Skeletal muscle regeneration

The next step of skeletal muscle recovery is the process of regeneration. During post-damage skeletal muscle regeneration, macrophages, which first exhibit an inflammatory profile, change towards an anti-inflammatory profile at the time of resolution of inflammation, therefore inflammatory cells play a dual role in promoting both injury and repair (Tidball, 2005). Inhibiting the anti-inflammatory signals during the second phase of muscle regeneration, as well as an alteration of the proinflammatory phase similarly leads to an impairment of muscle regeneration (Chazaud, 2016).

Macrophages play a key role in regeneration, which starts with the activation of satellite cells (Arnold et al., 2007), located between the basal lamina and sarcolemma (Hawke and Garry, 2001). Satellite cells are stem cells that stay quiescent in the skeletal muscle until damage occurs when they resume the cell cycle and proliferate. Proliferating satellite cells differentiate and grow, resulting in the formation of myoblasts, which later form new myofibers or fuse with existing myofibers to form repaired muscle tissue (Gharaibeh et al., 2012).

Skeletal muscle recovery is represented by a variety of multifactorial processes, which are jointly represented by the loss of muscle strength, increased muscle soreness and elevated blood markers of muscle damage and inflammation. Therefore, the majority of studies use a combination of markers to access the magnitude and pattern of muscle recovery (Damas et al., 2016). The rationale for using a variety of markers is that there should be at least one marker used to track the consequences of each of the physiological processes: 1) strength measurements to reflect extracellular matrix remodelling and failure in excitation-contraction coupling, 2) measurement of muscle soreness to reflect the damage in connective tissue and muscle swelling, 3) measurements of muscle proteins in plasma (CK and myoglobin) to reflect membrane damage and muscle protein leakage into bloodstream, 4) measurement of blood inflammatory markers to reflect the time course of inflammation (Damas et al., 2016).

2.2. Methods of assessment of skeletal muscle force, muscle soreness and muscle function

Skeletal muscle recovery is commonly defined as a return to baseline performance values. It can be assessed through a variety of parameters, including mechanical muscle damage, performance and related measures, muscle soreness, range of motion, muscle regeneration and inflammation (Markus et al., 2021). It does not appear to be a clear consensus in the literature as to which combination of methods produces the most reliable results (Warren et al., 1999).

2.2.1. Assessment of mechanical skeletal muscle damage

Mechanical skeletal muscle damage can be assessed by direct procedures, including invasive histological procedures (muscle biopsy) and non-invasive procedures [magnetic resonance imaging (MRI), ultrasound, and electromyography] (Markus et al., 2021). Muscle biopsies provide good insight into changes on the cellular level, including sarcomere disruptions and myofibrillar disruptions, the presence of inflammatory cells and fibres with centralized nuclei (indicative of regenerating fibres). However, they are not recommended for repeated measures due to the invasiveness of the procedure and related discomfort (Marqueste et al., 2008). MRI can detect changes in the skeletal muscle structure and oedema but cannot identify changes at the cellular level (Oudeman et al., 2016). Electron microscopy of a skeletal muscle biopsy reveals subcellular disorganisation of myofibrillar structure, especially in the Z-bands, and the presence of oedema (Paulsen et al., 2012). Ultrasound can identify fibre damage and disorganisation, pennation angle and oedema (Walker et al., 2004).

Several studies have observed the direct associations between changes in skeletal muscle's ability to generate force and histological observations of muscle damage, including myofibrillar disruptions, signs of necrosis and leucocyte accumulation, and changes in force-generating capacity seem to reflect myofibrillar disruption, inflammation and necrosis better than any other markers (Paulsen et al., 2012). However, others have noted a weak correlation between histological and functional measurements, including MVC torque measurements (Warren et al., 1999). Moreover, studies that have noted force reductions below 20%, have not found significant histological abnormalities (Bourgeois et al., 1999), whilst force reduction above 50% reported significant inflammation and myofiber necrosis (Fochi et al., 2016). Therefore, the use of histological or morphological assessments of structural muscle damage, while being an undisputed clinical tool for the identification of muscle injury, may not offer great insight into muscle contractile function during recovery.

2.2.2. Assessment of skeletal muscle performance

Skeletal muscle performance is accessed through a variety of methods, including measurement of muscle strength using MVC and/or 1-repetition maximum (1RM), vertical jump (VJ) or countermovement jump (CMJ), measurement of economy/efficiency and sprint tests (Markus et al., 2021). Measurement of muscle strength is considered to be the most relevant measure of muscle damage (Morton et al., 2005, Warren et al., 1999) and the most reliable quantifiable outcome of the loss of muscle function (Clarkson and Hubal, 2002). A review article by Paulsen et al., 2012 has summarized patterns of force recovery after eccentric exercise and has demonstrated that the pattern of recovery reflects the amount of muscle damage, with mild EIMD experiencing a 20% decline in force within 24 hours and full recovering in 48 hours, with moderate EIMD experiencing a 20-50% decline in force and recovering in 2-7 days, and with severe EIMD demonstrating more than a 50% decline in force and recovering in more than 1 week (Paulsen et al., 2012).

Strength is defined as peak force (N) or torque (N.m) developed under conditions of MVC (Abernethy et al., 1995). One of the most frequently assessed variables related to eccentric exercise is MVC torque, which is found to be directly proportional to the force (Warren et al., 1999). Important considerations for reliability of MVC torque measurements are angle and velocity, therefore torque should be ideally measured under isokinetic conditions (Warren et al., 1999). Studies estimated the validity of MVC torque measurements to be high, and the method is considered accurate and reliable (Abernethy et al., 1995, Kellis and Baltzopoulos, 1995). These studies found reliability coefficients for MVC torque measurements ranging from 0.85 to 0.99. For example, in one study, 20 young adults performed 5 hamstring MVC followed by contractions at 10-90% of MVC in a random order, with MVC repeated 1 week later. The reported test reliability between 2 test days was 0.95 (Campy et al., 2009). In another study, 10 healthy subjects performed sets of eccentric shoulder rotation (3 submaximal practice repetitions for 7 different angles), with a reliability of peak torque of 0.97 (Papotto et al., 2016). Studies found that reductions in MVC torque can be noticed throughout the whole process of skeletal muscle recovery, which makes this marker especially useful for the assessment of the timeline of muscle recovery (Saxton et al., 1995).

Common methods to measure skeletal muscle force include isometric, isotonic or isodynamic MVC or dynamic 1RM. Common equipment used for this purpose includes handheld dynamometry, seated strain gauge or computerised dynamometry (Machado Rodrigues et al., 2017).

Common methods to track strength recovery is represented by the measurement of peak force/torque using dynamometers (Gordon et al., 2017, Ferreira et al., 2017). Many studies acknowledge isokinetic dynamometers as the gold standard in the measurement of torque, with high reliability and validity of results (Ahmadi and Uchida, 2021, Drouin et al., 2004, McCleary and Andersen, 1992). Isokinetic dynamometers provide a stable velocity, with a resistance accommodated to applied force at a full range of motion (ROM) and allow accurate recordings of torque and power measurements (Drouin et al., 2004). The use of a dynamometer allows the minimization of measurement error to ensure observed changes in skeletal muscle function are due to changes in an individual’s performance, as opposed to inconsistent measurement techniques (Drouin et al., 2004).

Measurement of isometric force is often performed with computerised dynamometers, which are considered to be a “reference method”, however, they require high cost and space. Alternatively, isometric strain gauges offer a simple and low-cost solution to measure isometric force. Studies have found strong correlations between strain gauge measurements and similar isokinetic dynamometry measurements, with good validity and reliability (Machado Rodrigues et al., 2017).

1RM testing is commonly used but requires an intense effort to produce maximal output, and has an increased risk of injury and additional fatigue, which makes this marker not ideal for assessment of recovery (Raeder et al., 2016).

VJ height and flight time are often assessed in sports and have been shown to correlate positively with athletic performance (Sattler et al., 2012). Studies have also found a correlation between VJ performance and strength and power. One study has found a strong association between the strength of 64 weightlifters (men and women) with the estimated peak power from the VJ (Carlock et al., 2004). And another study has found significant correlations (0.726) between VJ peak power and VJ height of 12 male athletes performing 2 jumps (Nuzzo et al., 2008). Some of the criticism of using VJ as a measure of strength is that VJ utilizes a combination of muscular strength of the lower body and body coordination, so the performance is not truly dependent on muscular strength alone, therefore VJ can be used as an indirect index of power and strength (Hackett et al., 2018). VJ has demonstrated good test-retest reliability in a few studies. For example, reliability was 0.98 in a study by Hackett et al., 2008 testing for correlation between power peaks of VJ with other performance measures (medicine ball chest throw) between 3 trials of 190 adolescent participants.

A variety of methods used to assess the VJ height, including jump mats, were found to have high accuracy and reliability of results (Montoye and Mitrzyk, 2018). The study by Aragon-Vargas, 2000 evaluated four different methods of measuring VJ, including time in the air calculated by jump mats. The correlation coefficient for 5 vertical jumps was 0.994 and the validity coefficient is 0.95. Electronic jump mats provide some advantages over traditional VJ, including improved accuracy and precision, efficiency and shorter testing times, automatic calculation of jump height based on the flight time, and ease of transportation and storage (Klavora, 2000). The same study has noted that to ensure measurement accuracy and reliability, it is important to ensure a consistent landing technique, where participants land in the same position they took off.

The countermovement jump (CMJ) protocol used in this study is an example of VJ, which is derived from the Sargent jump (Sargent, 1924). The CMJ is characterized by an initial countermovement before the jump (Bobbert et al., 1996), and the CMJ provides reliable information about the strength of the lower limbs (Young, 1995, Markovic et al., 2004). In this study, physical education students performed 7 explosive power tests: Sargent jump, Abalakow's jump with arm swing and without arm swing, CMJ, and 2 horizontal jumps (standing long jump and standing triple jump). CMJ had the greatest reliability among all jumping tests and showed the highest relationship with the explosive power factor.

2.2.3. Assessment of skeletal muscle soreness

DOMS manifest in sensations of dull pain associated with movements or pressure at worked skeletal muscle. It typically develops within 24 hours after exercise, reaching a peak within 1-3 days after exercise (Cheung et al., 2003). Muscle soreness is associated with muscle stiffness, increased sensitivity and local pain sensations (Lewis et al., 2012) and it is one the most commonly used markers of skeletal muscle damage (Warren et al., 1999). Soreness is not always easy to assess and quantify due to its subjective nature (Lau et al., 2013b). Soreness can be assessed through objective measures, such as measuring the pressure applied at the muscle at the point threshold (Warren et al., 1999), or by using visual pain scales evaluating pain intensity, including unidimensional visual analogue scales (VAS) and the multidimensional McGill Pain Questionnaire (Markus et al., 2021).

VAS is represented by a continuum, where one side indicates no pain, and the other side represents the worst pain. VAS is one of the most common methods of pain assessment, and it is considered to be a reliable measure of skeletal muscle pain after exercise (Boonstra et al., 2008). Apart from measuring generalised pain after exercise, VAS can be used to measure pain in specific muscles affected by exercise (Thompson et al., 1999). In a study by Lau et al., 2013a 31 subjects performed 10 sets of 6 maximal eccentric contractions of the elbow flexors. Measurements were repeated on 4 days for VAS (100 scale) and Pressure Pain Threshold (PPT) taken with an algometer. The intraclass correlation coefficient (ICC) for VAS measurements was 0.98-0.99 and ICC for PPT was 0.92-0.98, demonstrating good reliability for both assessment methods.

Another reliable method of quantifying pain is to measure PPT with a pressure algometer (Jones et al., 2007). A pressure algometer measures the point where an applied pressure to the working skeletal muscle turns into a sensation of pain. Several studies have tested the reliability of PPT in different patterns of repetitions, including 10 to 50 sequential measurements, and concluded that there were no significant differences between trials (Nussbaum and Downes, 1998). For example, in a study by Chesterton et al., 2007, 3 measurements were taken for each of 13 participants by 5 evaluators with an ICC of 91%, indicating high reliability of results. This study also mentioned the importance of applying consistent force and the same application angle for higher results reliability.

Studies have found that PPT has a different time course compared to VAS: it decreases the most one day after the exercise and remains decreased till the end of recovery (Lau and Nosaka, 2011). Studies often access soreness using PPT in combination with VAS, as it assesses two different aspects of pain. PPT measures the minimum pressure required to induce pain in skeletal muscle and focused on muscle sensitivity while VAS measures the magnitude of pain after the exercise and is focused on pain intensity (Lau et al., 2013b). Studies have found a weak correlation between measurements of soreness and functional and histological measurements of skeletal muscle damage (Warren et al., 1999).

2.2.4. Use of blood markers to access the skeletal muscle recovery

Blood markers are widely used to evaluate skeletal muscle damage and understand the recovery status from EIMD, including creatine kinase (CK) and myoglobin (Mb), and inflammatory markers (Baird et al., 2012, Bessa et al., 2016). Clinically significant amounts of CK are found in skeletal muscle and its specific isoform is referred to as muscle isoform of creatine kinase (CK-MM). Serum CK-MM levels are physiologically quite low and increase significantly after EIMD and are widely used in the literature as a marker of muscle skeletal damage (Castro et al., 2011, Brancaccio et al., 2006, Ehlers et al., 2002). Peak CK-MM is reached in 24-48 hours and can still be present 72 hours after exercise. (Brancaccio et al., 2006, Ferri et al., 2006). It has been indicated that the use of CK-MM alone may not always provide an accurate picture and should be used in conjunction with other assessments (Magal et al., 2010). Some studies have demonstrated that levels of CK may be affected by hydration status (Fielding et al., 2000). One of the suggested explanations for decreased accuracy of CK-MM as a marker of damage is the significant individual variations of CK-MM, with some athletes being non-responders (Halson, 2014). Studies have found a weak correlation between functional markers (MVC torque) and blood levels of CK-MM and histological markers (Warren et al., 1999). Others found that when exercising at a lower intensity (60% of MVC), functional markers (MVC torque) correlate with levels of CK, oedema and pain sensations, compared to 80% of MVC, where correlations were not significant (Baird et al., 2012).

2.2.4.1. Inflammation markers

Inflammatory markers can provide a fast and accurate assessment of skeletal muscle damage and can indicate the recovery stage (Bessa et al., 2016). Many studies have found an increase in concentrations of IL-6, IL-8, IL-10, and TNF-α after 1 day following exercise (Pedersen and Febbraio, 2008, Dorneles et al., 2016, Suzuki et al., 2000).

Plasma concentrations of IL-6, which has both pro-inflammatory and anti-inflammatory functions, appear straight after exercise, peak after 1 hour and can increase up to 100-fold. Therefore, this cytokine is often used as an inflammation marker as it is easy to detect and precedes the appearance of other cytokines (Ostrowski et al., 1998b). A review by Cerqueira et al., 2019 reported exercise-induced increases in IL-6 from 1.33 to 4.20 times in moderate and from 1.59 to 26.79 times in high-intensity exercise.

Other cytokines, that were found to increase after exercise, are IL-8 and IL-10. A review by Cerqueira et al., 2019 found a 1.37 to 2.77 times increase in IL-8 both after moderate and intense exercise. A study by Ostrowski et al., 1998a found a 27-fold increase in IL-10 immediately post-exercise (marathon).

Many studies only found TNF-α to be increased after prolonged strenuous exercise (Pedersen and Febbraio, 2008). A study by Ostrowski et al., 1998a found a 25% increase in levels of TNF-α 3 hours after the exercise, and at 6 hours post-exercise. However, by day 2 and 5, the levels were 15% below the pre-exercise levels.

Based on the above, measuring the levels of inflammatory markers can help in understanding the stage of the recovery process, as peaked levels of IL-6 and TNF-α are indicative of the pro-inflammatory stage and peaked levels of IL-8 and IL-10 are indicative of the anti-inflammatory and recovery stages.

2.3. Factors that have an impact on skeletal muscle recovery

Skeletal muscle recovery is an important part of any competitive sports programme (Castro et al., 2011), and several factors have been investigated in the literature to see whether they have an impact on muscle recovery, including type, intensity and duration of exercise, presence of previous muscle damage, muscle groups used, torque and the number of contractions, genetics and nutrition (Markus et al., 2021).

The most well-researched factor that influences skeletal muscle recovery is a presence of previous muscle damage, referred to as the “repeated bout effect”. After an initial bout of muscle-damaging exercise, the muscle goes through structural and metabolic adaptations, so that symptoms of EIMD are less pronounced at the consequent bouts of exercise (Peake et al., 2017). Structural muscle adaptations include extracellular matrix remodelling of intermediate filaments and the addition of sarcomeres, leading to longer fibres to protect the muscle against future damage (Baumert et al., 2016).

The time course of recovery after EIMD depends on the extent of damage, which is determined by the type, intensity, duration, and repetitions of the exercise performed, and type of the skeletal muscle used (de Azevedo Franke et al., 2021, Tomlin and Wenger, 2001). Studies investigating the impact of the type of exercise have reported that eccentric exercise, which involves muscle lengthening under tension, results in longer periods of recovery and higher inflammatory responses compared to concentric or isometric exercise (Peake et al., 2017). High- *vs.* low-force muscle contractions are more likely to create greater damage to contractile proteins and create more severe EIMD (Nosaka and Newton, 2002). A large number of fast *vs.* slow velocity eccentric muscle contractions produce greater force at longer lengths and activate fewer cross bridges, which increases the risk of damage to contractile proteins and results in more severe EIMD (Chapman et al., 2008).

Other factors that have been investigated to influence skeletal muscle recovery include sex, training experience, age, and amount of sleep. In studies analysing sex differences in muscle recovery, females demonstrated longer recovery following a single bout of resistance exercise compared to males (Sayers and Clarkson, 2001). Longer periods of muscle recovery were demonstrated by amateur athletes compared to experienced athletes (Magalhães et al., 2018), and under sleep-deprived conditions (Dáttilo et al., 2020). There is evidence that older people experience longer periods of muscle recovery due to impaired muscle regeneration and remodelling (Fell and Williams, 2008).

Some studies found there are individual variations related to EIMD and that subjects with certain genotype experience more skeletal muscle damage than others and have a longer recovery period following strenuous exercise. Baumert et al., 2016 have concluded that individuals with certain genotypes experience greater muscle damage and require longer recovery following strenuous exercise. These polymorphisms include ACTN3 (R577X, rs1815739), TNF (−308 G>A, rs1800629), IL6 (−174 G>C, rs1800795), and IGF2 (ApaI, 17200 G>A, rs680). This is a promising area of research. Knowing how an individual responds to exercise can help to individualise training and maximise recovery and adaptation (Baumert et al., 2016).

2.3.1. Impact of nutrition on skeletal muscle recovery

There is emerging evidence in the literature that nutritional interventions have the potential to improve recovery from EIMD (Harty et al., 2019). Recent trends in nutritional epidemiology include moving away from analysing a single element in the diet to the holistic approach of analysing the impact of dietary patterns (Craddock et al., 2019). There has been a limited number of studies that have examined the impact of certain diets on the recovery of skeletal muscle function, and even fewer studies that have attempted to compare the impact of various diets (Markus et al., 2021).

Plant-based diets (including vegan diets) are becoming popular among the general population and athletes (Wirnitzer et al., 2016). Veganism is defined as "*a philosophy and way of living which seeks to exclude – as far as is possible and practicable – all forms of exploitation of, and cruelty to, animals for food, clothing or any other purpose; and by extension, promotes the development and use of animal-free alternatives for the benefit of animals, humans and the environment. In dietary terms, it denotes the practice of dispensing with all products derived wholly or partly from animals*" (The Vegan Society, 2021). In contrast, an omnivore diet is defined in the literature as consuming products of animal origin and their derivatives at least three times per week (Menzel et al., 2020).

2.3.2. Nutrient composition of a vegan diet compared to an omnivorous diet

Studies note that vegan diets are considered to be low in branch-chain amino acids, creatine, and omega-3 fatty acids due to a lack of intake of meat, eggs, fish and seafood (Vitale and Hueglin, 2021). All these nutrients have been demonstrated to be beneficial for skeletal muscle recovery, though there are no studies that have proven that muscle recovery will be impaired when those nutrients are absent in the diet. At the same time, a vegan diet often has higher carbohydrate content, which may promote better glycogen storage, important for athletic performance (Barnard et al., 2019). Typically a vegan diet has lower fat content, especially omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) due to a lack of eggs or seafood (Vitale and Hueglin, 2021). The plant-based nature of vegan diets also makes them lower in saturated fat and higher in fibre content (Lindqvist et al., 2021). Because of the high fibre intake and low caloric density of plant-based foods, a vegan diet is associated with a higher risk of inadequate energy intake. Athletes not meeting their energy intake may experience fatigue and delayed recovery (Thomas et al., 2016). A vegan diet may be considered to be deficient in some micronutrients, including calcium, iron and vitamin B_{12}, vitamin D (Clarys et al., 2014, Schüpbach et al., 2017), and iodine (Eveleigh et al., 2020). An omnivorous diet may be low in magnesium, vitamins C and E, niacin and folic acid, but has a higher protein content (Schüpbach et al., 2017).

2.3.3. Health benefits associated with a vegan diet compared to an omnivorous diet

There is a lot of research on various health benefits associated with vegan diets in comparison with omnivorous diets, including reduced body mass index (BMI), lower risk of diseases, including metabolic syndrome, cardiovascular diseases, hypertension, hypothyroidism and some cancers, and increased longevity (Table 2.1).

Table 2.1. Health benefits associated with a vegan diet compared to an omnivorous diet

N	Authors	Description	Outcome
1	Goff et al., 2005	Compared biochemical profiles of vegan *vs.* omnivores matched for age, gender and body mass index (BMI)	Vegan had better cardioprotective markers: lower blood pressure and fasting triglycerides and glucose concentrations

N	Authors	Description	Outcome
2	Fontana et al., 2007	Health characteristics of long-term vegans compared to the group following the Western diet (omnivores)	Vegans demonstrated reduced BMI and cardioprotective markers: lipids, lipoproteins, glucose, insulin, C-reactive proteins (CRP), blood pressure and carotid artery thickness
3	Rizzo et al., 2013	The difference in nutrient profiles between vegetarian[1] and non-vegetarian dietary patterns	Lowest BMI in vegetarians[1] compared to all other diets
4	Tonstad et al., 2009	Prevalence of type II diabetes between different types of vegetarian[1] and non-vegetarians' diets	Vegetarians[1] demonstrated the lowest BMI and reduced risk of developing type II diabetes
5	Wirnitzer et al., 2019	Health parameters of vegan *vs.* omnivore endurance runners	Vegan athletes appear to be more health conscious
6	Tonstad et al., 2013	Risk of hypothyroidism of vegan *vs.* omnivore participants	Vegan group demonstrated a lower risk of hypothyroidism compared to the omnivore group
7	Le and Sabate, 2014	Effects of vegan diets on health and disease outcome	Vegans demonstrated lower BMI, lower risk of coronary heart disease risk, hypertension, type II diabetes, metabolic syndrome, and some types of cancers, as well as greater life expectancy
8	Menzel et al., 2020	Systematic review and meta-analysis of the associations of a vegan diet with inflammatory markers compared to the omnivores	Vegan group demonstrated lower levels of CRP compared to omnivore group. No pronounced effects were observed for other inflammatory markers (IL-1, IL-6, IL-8, TNF-α)

[1] No consumption or consuming less than once a month: meat (red and poultry), fish, eggs, milk, and dairy.

At the same time, a recent systematic review by Bakaloudi et al., 2021 of vegan diets has indicated that a typical vegan diet is associated with a deficiency of some micronutrients, including calcium, iron, vitamin B2, vitamin B3 (niacin), vitamin B_{12}, vitamin D, zinc, calcium and iodine. Vitamin deficiencies should not be disregarded, though they are not always associated with health impairments.

One of the components of good health is diet quality (Wirnitzer et al., 2018). But do health benefits translate to improved skeletal muscle recovery? Due to the high diversity of dietary intake in vegan diets, there are no high-quality studies on the effect of vegan diets on athletic performance and function, including muscle recovery (Vitale and Hueglin, 2021).

Vegan diets may have a lower intake of certain nutrients that have been hypothesized to have a positive impact on the recovery of skeletal muscle function, including, protein, branch-chained amino acids, creatine and omega-3 fatty acids. Creatine supplementation has been found to enhance recovery from exercise by reducing muscle damage and inflammatory markers (Kreider et al., 2017). In vegan athletes, creatine stores can be lower due to the lack of intake of animal-based food, containing creatine. This has the potential to affect their performance at high-intensity exercise (Kaviani et al., 2020). Studies have demonstrated that omega-3 supplementation may reduce the effects of exercise-induced muscle damage, especially in a higher dose (Xin and Eshaghi, 2021, Visconti et al., 2021). One of the potential consequences of a lack of animal-based protein in the diet is the lack of vitamin B_{12}, which may potentially result in reduced production of red blood cells, monocytes, leukocytes, neutrophils and platelets (Anitha et al., 2014, Lederer et al., 2020).

On the other hand, there are a few main properties of vegan diets that could have a positive impact on skeletal muscle recovery (Craddock et al., 2016), including a high intake of foods rich in antioxidants, polyphenols and other phytochemicals (Melina et al., 2016), considered to be anti-inflammatory compounds. While inflammation causes certain muscle damage, it is an integral part of regeneration and recovery, and it was hypothesized that factors that blunt inflammatory response can potentially result in longer muscle recovery (Urso, 2013). It is still uncertain how vegan diets could influence the inflammatory response of muscle recovery, but it is possible they could modulate inflammation and tissue regeneration. A study has compared skin healing after dermatological treatments for a skin condition (actinic keratosis) between vegans and omnivores and found that the vegan group had a longer recovery period and a larger incidence of side effects (Fusano et al., 2021).

2.3.4. Phytochemicals, flavonoids and polyphenols

As the post-exercise state is characterized by the inflammatory response, it was hypothesized that increased phytochemical intake from plant sources can attenuate the increased exercise-induced oxidative stress (Trapp et al., 2010, Vitale et al., 2017). There are several studies analysing the impact of polyphenols on skeletal muscle recovery, in particular flavonoids, the bioactive compounds found in fruits, teas and coffees. The majority of studies agree that supplementation with polyphenols containing flavonoids can improve the recovery of muscle strength and reduce muscle soreness after exercise-induced muscle damage through the reduction of oxidative stress (Carey et al., 2021, Rickards et al., 2021, Clifford et al., 2016). However, the bioavailability of flavonoids consumed through food is not high enough to achieve the concentrations necessary to produce the noticeable impact, with less than 5% of phenolic compounds reaching the bloodstream, and much of the remaining 95% being fermented by the gut (Clifford, 2004).

A study comparing the oxidative stress response after graded exercise tests performed until voluntary exhaustion on a bicycle ergometer between omnivores and vegans has found increased markers of oxidative stress and lipid peroxidation in vegans (Nebl et al., 2019). This was explained by the higher creatine content of the omnivore diet, which also has antioxidant properties. Another study comparing resting oxidative stress markers between vegans and omnivores has found that the total antioxidant status of plasma was lower in the vegan group and lipid peroxidation levels increased in vegan group (Vanacore et al., 2018). This was explained by the high fibre content of the vegan diet, which lower the polyphenols availability, resulting in increased levels of oxidative stress. However, there is no agreement that the antioxidant properties of the vegan diet would translate to an advantage in terms of recovery from EIMD.

2.3.5. Protein and branch-chain amino acids

The role of protein in skeletal muscle recovery has been widely investigated in the literature and is always present in the nutritional recommendations for athletes (Leitzmann, 2014). Studies have demonstrated that consuming protein supplements during recovery from exercise stimulates muscle anabolism (Arentson-Lantz and Kilroe, 2021) and increases rates of protein synthesis (Breen et al., 2011). This promotes skeletal muscle remodelling and therefore facilitates long-term muscle recovery (Churchward-Venne et al., 2020). Though improved muscle anabolism with protein supplementation should theoretically hasten muscle recovery, it has not resulted in significant reductions in muscle damage and enhanced recovery in studies.

A review by Pasiakos et al., 2014, analysing 27 studies on protein supplementation and skeletal muscle recovery, has not found an association between recovery of muscle function and measurable markers of muscle damage and soreness when protein supplements are taken before, during or after the bout of exercise (Pasiakos et al., 2014). However, it was outlined that the impact of protein supplementation on muscle recovery is more pronounced when participants are in negative nitrogen and/or energy balance (Pasiakos et al., 2014). After unaccustomed exercise, muscle damage triggers increased rates of muscle protein synthesis and degradation and, in the absence of adequate protein intake, it can result in a negative protein balance (Levenhagen et al., 2002). Even though a vegan diet contains protein, plant-based proteins have lower digestibility and bioavailability, which can result in negative nitrogen balance during an intensive exercise regimen (Hoffman and Falvo, 2004). A recent review has analysed 29 studies and 40 trials to conclude that peri-exercise protein consumption could help maintain maximal strength and lower CK concentration following resistance exercise but does not reduce skeletal muscle soreness (Pearson et al., 2022).

The effects of branch-chain amino acids on skeletal muscle recovery are under debate in the literature. Some studies have demonstrated that the ingestion of branch-chained amino acids (BCAA) after exercise can reduce muscle soreness (Howatson et al., 2012, Weber et al., 2021), but it is still uncertain how that reflects on force recovery. A systematic review by Doma et al., 2021b has concluded that BCAA reduces the level of creatine kinase and muscle soreness following strenuous exercise with a dose-dependent relationship but does not accelerate the recovery for muscle performance. Similarly, Fedewa et al., 2019 showed that BCAA supplementation reduced DOMS following exercise training. However, Rahimi et al., 2017 concluded that BCAAs significantly reduce creatine kinase at two follow-up times (<24 and 24 h) in comparison with placebo recovery, but effects were not significant in any of the follow-up times for muscle soreness. Moreover, some other studies have not reported any significant impact of branch-chained amino acids on recovery (Ra et al., 2018) or found effects dependable on type, dosage and administration protocol (Fouré and Bendahan, 2017).

CHAPTER 3.
RESEARCH STUDY MANUSCRIPT

3.1. Abstract

Purpose: The purpose of the study was to compare the recovery of skeletal muscle function post-strenuous exercise between healthy young males on a vegan diet with healthy young males on an omnivorous diet.

Methods: Six vegan (33±6 years, 75±13 kg, 1.79±0.06 m, 23.13±2.82 kg/m^2) and six omnivore (27±7 years, 84±12 kg, 1.77±0.07 m, 27.07±4.77 kg/m^2) healthy males performed an established muscle-damaging exercise protocol, consisting of 5 sets of 20 drop jumps from a 0.6 m high platform. Skeletal muscle function was evaluated using double-legged countermovement jumps (CMJ). Generalized pain at rest and during CMJ was accessed with a visual analogue pain scale (VAS) and muscle-specific soreness at rest was assessed by asking participants to verbally rate the level of pain experienced in specific muscles of each leg. Pressure pain threshold (PPT) was used as a secondary measure of muscle-specific soreness, using a handheld algometer. These parameters were measured before drop jumps and at specific time points (0 h, 1 h, 3 h, 24 h, 48 h and 72 h following the drop jumps). 4-day food diaries were collected to access participants' habitual diets.

Results: There was no significant difference in CMJ height over time points (F=4.223, P=0.370) across the study, and diet type also had no significant effect on CMJ height (F=0.820, P=0.786). There was a significant increase for generalised pain at rest (F=10.868, P=0.002) and during CMJ (F=8.428, P=0.003), observed over time points, which was similar between diet types. Subjective pain at specific muscles at rest was also mostly similar between vegans and omnivores, except for glutes left leg (F=12.377, P=0.017) and inner thigh left leg (F=10.741, P=0.022), where vegans had less pain. PPT was not affected by the exercise protocol and was similar between vegans and omnivores, except for the calf lateral head left leg where omnivores had a slightly lower PPT (F=10.988, P=0.021).

Conclusion: The study found no differences in recovery of skeletal muscle function between omnivores and vegans, despite sporadic differences in muscle soreness. Mechanisms linking habitual diet and delayed-onset muscle soreness (DOMS) are still unclear, and the results of our study should be confirmed by larger or more powerful studies.

3.2. Introduction

Athletes require a significant amount of training to achieve a competitive advantage in sport and optimal recovery is essential to any exercise and fitness programme (Castro et al., 2011). Strenuous exercise often results in exercise-induced muscle damage (EIMD) (Doma et al., 2021a). EIMD is associated with oxidative stress and inflammation, muscle soreness and reduction in muscle function (Corr et al., 2021). Several studies have indicated that EIMD can harm the quality of training and overall performance in sports (Doma et al., 2021a). Skeletal muscle recovery is an important part of any competitive training schedule (Castro et al., 2011), and investigating the factors that affect recovery is relevant.

Several factors have been investigated to see whether they have an impact on skeletal muscle recovery including age, training experience (Zauner et al., 1989), gender, amount of sleep (Castro et al., 2011), type of exercise (Tomlin and Wenger, 2001), and nutrition (Pasiakos et al., 2014). Eccentric exercise results in higher inflammatory response and the need for a longer period of recovery compared to concentric exercise (Peake et al., 2017). Some studies noted that an important factor that influences muscle recovery is the presence of previous muscle damage, as the first bout of exercise produces a stronger response compared to further exercise bouts due to the muscle adaptions (Peake et al., 2017). A minority of studies reported some individual differences in responding to muscle damage between the participants (Damas et al., 2016). This study was measuring changes in MVC, skeletal muscle soreness, CK activity, range of motion (ROM) and upper-arm circumference before and for several days after unaccustomed maximal eccentric exercise bout of the elbow flexors. The causes of those individual differences were not fully explained yet but may have a genetic background (Baumert et al., 2016).

Certain aspects of the possible effect of nutrition on the recovery of skeletal muscle function have been investigated, including protein (Pasiakos et al., 2014), branch-chained amino acids (Howatson et al., 2012), flavonoids (Carey et al., 2021), omega-3 polyunsaturated fatty acids (Smith et al., 2011), vitamin D (Barker et al., 2013). However, recent trends in nutritional epidemiology move away from analysing a single element in the diet to a holistic approach to analysing the impact of dietary patterns (Craddock et al., 2016). Plant-based diets are becoming increasingly popular all over the world, in particular the vegan diet, in which all food sources of animal origin and derivatives are avoided, including meat, poultry, fish, eggs and dairy products. In contrast, omnivore diets are defined as diets including habitual consumption of animal sources of protein at least once a week (Rizzo et al., 2013). Due to the high diversity of the dietary intake in vegan diets, there are no high-quality studies on the effect of a vegan diet overall on athletic performance (Vitale and Hueglin, 2021).

There are a few main properties of vegan diets that could have a positive impact on skeletal muscle recovery (Craddock et al., 2016), including a high intake of foods rich in antioxidants, polyphenols and other phytochemicals (Melina et al., 2016), considered to be anti-inflammatory compounds. Increased phytochemicals and antioxidants abundant in vegan diets may help to reduce oxidative stress associated with prolonged exercise, therefore potentially enhancing muscle recovery. However, the link relating a vegan diet to exercise-induced oxidative stress is inconclusive with many of the studies focusing on supplementations (Trapp et al., 2010).

To date, human studies investigating and comparing the impact of a vegan *vs.* an omnivore diet on skeletal muscle recovery are sparse. Therefore, the main purpose of the study was to establish whether there is a difference in the recovery of muscle performance and soreness between vegans and omnivores following a muscle-damaging exercise in the form of 100 drop jumps.

3.3. Methods

3.3.1. Participants

Promotional materials were distributed through social platforms (vegan and community groups on social media), and promotional presentations were given to Massey University students before lectures and posters were distributed on the Auckland campus. Participants were included in the study if they met the following criteria: (1) male, (2) aged between 18–45 years old and (3) have adhered to either a vegan or an omnivore diet for at least the last 2 years. Exclusion criteria were: (1) chronic diseases such as cardiovascular disease and diabetes, (2) prescription medication and/or anabolic steroids that may interfere with inflammatory biomarkers or physical ability, (3) cigarette smoker, (4) orthopaedic limitations or musculoskeletal disorders, (5) involved in heavy strength training or other high-force activities, including near maximal or maximal eccentric force generation.

Initially, 65 participants expressed their interest to participate in the study, 39 vegans and 26 omnivores. Subjects who expressed interest received an information sheet with further study details (Appendix B.1.). 18 participants had not responded after receiving additional information about the study, and 13 participants declined participation after receiving additional information due to various reasons, including concerns about the blood being taken multiple times, unwillingness to stop taking supplements and/or training during the trial, other time commitments. All remaining participants were screened with a Health Screening Questionnaire (Appendix B.2.) to eliminate any potential health risks and to address the exclusion criteria. The Health Screening Questionnaire included questions on habitual dietary intake to assist allocation into the appropriate experimental group. 11 volunteers did not meet the study criteria due to various reasons, including taking medications, which can impact the study results, and doing resistance training regularly. Participants were accepted over a familiarization trial, when they were further informed about the purpose of the study, and potential risks involved, and were asked to give written consent before participation (Appendix B.3.). After being accepted, a further 11 participants could not participate due to sickness and other commitments. As a result, 6 vegan and 6 omnivore healthy males were recruited into the study (Figure 3.1.). This study has received approval from Massey University Human Ethics Committee: Southern A, Application SOA 22/10.

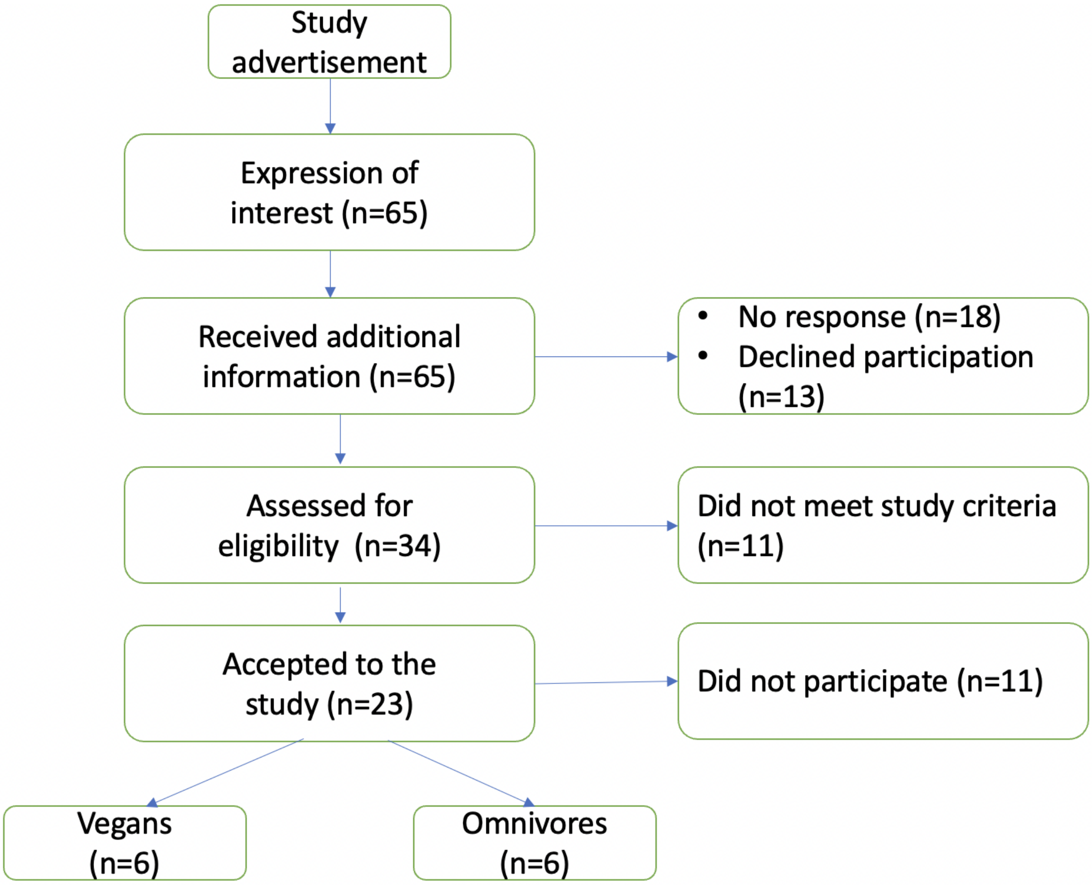

Figure 3.1. CONSORT recruitment flowchart

3.3.2. Study design

Before the main trial, participants attended a familiarisation visit, where they tried the exercise protocol using the drop jumps. Participants also performed the countermovement jump (CMJ) protocol, and measurements of soreness were taken with the pressure algometer and with generalized and specific pain scales (0-10).

Participants were asked to complete a four-day food diary (Appendix A.1.) 3 days before the experimental period and on the first test day to assess dietary intake, as well as to maintain their habitual diet through the duration of the study and to abstain from exercise for 48 h before the start of the exercise protocol session until the completion of the assessments. Additionally, participants could not consume any alcohol, caffeine, or dietary supplements 24 h before, or during the experimental period.

The following baseline measurements were performed on the intervention day: (1) weight, (2) height, (3) measurement of skeletal muscle function (double-legged CMJ), (4) muscle soreness was accessed with pressure algometer and generalized and muscle-specific pain scales. All baseline measurements (excluding weight and height) were repeated at 0 h, 1 h, 3 h, 24 h, 48 h and 72 h following the drop jumps (Table 3.1.).

Table 3.1. Study design procedures. W&H: weight and height. DJ: drop jumps. CMJ&GPS: countermovement jumps and generalized pain scales. PA: pressure algometer. SPS: specific pain scales. GPS: generalized pain scales. FD: food diary.

Familiarisation visit	DAY 1					DAY 2	DAY 3	DAY 4
	Baseline	Protocol	0 h	1 h	3 h	24 h	48 h	72 h
W&H		DJ						
GPS	GPS	GPS	GPS	GPS	GPS	GPS	GPS	GPS
SPS	SPS		SPS	SPS	SPS	SPS	SPS	SPS
PA	PA		PA	PA	PA	PA	PA	PA
CMJ	CMJ		CMJ	CMJ	CMJ	CMJ	CMJ	CMJ
			FD					

3.3.3. Experimental methods

3.3.3.1. Damage-inducing exercise protocol

For the study intervention, participants were asked to perform an established skeletal muscle-damaging exercise protocol consisting of 100 drop jumps from a 0.6 m-high platform. The protocol has been demonstrated to establish a high level of exercise-induced muscle damage and subsequent soreness and is described elsewhere in more detail (Clifford et al., 2016). Participants performed 5 sets of 20 drop jumps. Each jump was separated by a 10 s interval and each set was separated by 2 minutes rest, with water permitted to be consumed *ad libitum*. Participants were instructed to drop from the box and land on both feet, descend to ~90° knee flexion and immediately perform a maximal vertical jump. To ensure the participant completed a maximal effort, a Styrofoam ball was suspended from the ceiling at the appropriate maximal reach-height for the individual, as determined during the familiarisation visit. Participants were asked to reach the ball on each repetition. Furthermore, during the exercise protocol, participants were verbally encouraged to produce a maximal effort.

3.3.3.2. Skeletal muscle soreness

A validated paper-based visual analogue pain scale (Delgado et al., 2018) was used as a subjective measure for overall skeletal muscle soreness. Participants were asked to indicate the level of generalised pain experienced in both legs at rest and after the CMJ protocol according to a 10-point continuous scale representing no pain (score 0) and worst pain (score 10). Measurements were at baseline and at specific time points: 0 h, 1 h, 3 h, 24 h, 48 h and 72 h following the drop jumps. Muscle-specific soreness was assessed at rest, over the same time points, by asking participants to verbally rate the level of pain experienced in specific muscle groups of each leg (gastrocnemius, gluteus maximus, hamstrings, inner thighs, outer thigh, tibialis), using the same scale 10-point scale.

Pressure pain threshold (PPT) was used as a secondary measure of muscle-specific soreness, using a handheld algometer (Force Dial FDK 60, Wagner Instruments; Greenwich, CT, USA) at baseline and at specific time points (0 h, 1 h, 3 h, 24 h, 48 h and 72 h) following the drop jumps. Participants were asked to remain seated, while a 1 cm-diameter cylindrical flat-headed probe was used to apply pressure to pre-marked muscle points on each leg (a mid-portion of the vastus lateralis, rectus femoris, vastus medialis, biceps femoris, semitendinosus, gastrocnemius lateral head and gastrocnemius medial head). The participant was asked to signal when they felt pain or discomfort, at which point the algometer PPT value was recorded as load (kg/cm^2). The pressure was applied at a constant rate of 10 N cm^{-2}·s^{-1} as previously described (Pelfort et al., 2015).

3.3.3.3. Skeletal muscle function

Skeletal muscle function was evaluated using double-legged countermovement jumps (CMJ) on a jumping mat (Just Jump System, Probotics; Huntsville, AL, USA) at baseline and at specific time points (0 h, 1 h, 3 h, 24 h, 48 h and 72 h). To perform the jumps, participants were asked to stand on the jump mat with both feet flat on the ground at shoulder width apart and hands on hips. Participants were asked to start from a vertical position, then drop into a semi-squatting position with knees at ~90° knee angle, and immediately countermove upwards into a jump with maximum force. Participants were instructed to land on the mat with both feet, keeping their hands on their hips for the full movement to minimise any influence of arm swing. Three maximal efforts were performed, with a 30 s rest between jumps. Jump height (m) was recorded and the mean height was calculated for analysis to reduce the impact of jump technique on jump performance.

3.3.4. Statistical Analysis

Data were analysed using the SPSS Statistics software version 25 (IBM, Armonk, NY, USA). Repeated measures analysis of variance (ANOVA) was applied to test for effects of diet type (vegans *vs.* omnivores), time points (baseline, 0h, 1h, 3h, 24h, 48h and 72 hours post-exercise) and interactions between diet type and time points for each dependent variable, i.e., jump height, algometer measurements and skeletal muscle soreness ratings. Values are represented as means ± standard deviation at a 95% confidence level. Tendency to significance was considered for values of P between 0.05-0.1. Mauchly's test of sphericity was carried out to test whether the assumption of sphericity is met in a two-way repeated-measures ANOVA. When we found a significant difference in ANOVA, we did a Bonferroni post-hoc test.

3.4. Results

The participants' characteristics are described in the table below (Table 3.2.):

Table 3.2. Participants' baseline measures

	Vegan (Mean±SD)	Omnivores (Mean±SD)	P-value
Age (years)	33±6	27±7	0.166
Weight (kg)	75±13	84±12	0.213
Height (m)	1.79±0.06	1.77±0.07	0.612
BMI (kg/m^2)	23.1±2.8	27.1±4.8	0.112
PPT right leg, vastus lateralis (kg/cm^2)	7.4±2.3	5.8±1.8	0.208
PPT right leg, rectus femoris (kg/cm^2)	8.8±3.4	6.2±1.8	0.134
PPT right leg, vastus medialis (kg/cm^2)	9.1±3.5	6.5±2.4	0.161
PPT right leg, biceps femoris (kg/cm^2)	8.3±1.3	8.0±3.0	0.838
PPT right leg, semitendinosus (kg/cm^2)	8.1±1.9	6.9±4.4	0.552
PPT right leg, gastrocnemius lateral head (kg/cm^2)	7.3±1.9	7.0±1.9	0.792
PPT right leg, gastrocnemius medial head (kg/cm^2)	7.2±1.6	5.1±1.5	*0.035
PPT left leg, vastus lateralis (kg/cm^2)	7.4±1.9	6.4±2.7	0.494
PPT left leg, rectus femoris (kg/cm^2)	8.7±3.0	7.2±2.7	0.381
PPT left leg, vastus medialis ((kg/cm^2)	9.1±3.0	6.7±2.3	0.161
PPT left leg, biceps femoris (kg/cm^2)	8.2±2.1	6.9±2.5	0.322
PPT left leg, semitendinosus (kg/cm^2)	8.8±1.9	5.6±2.9	*0.005
PPT left leg, gastrocnemius lateral head (kg/cm^2)	7.8±2.1	6.1±2.3	0.200
PPT left leg, gastrocnemius medial head (kg/cm^2)	6.8±1.8	5.7±2.6	0.398
CMJ height (m)	0.44±0.08	0.42±0.06	0.600

* Significant difference calculated by unpaired two-tailed t-test.

3.4.1. Countermovement jumps (CMJ)

Even though there was a slight decrease in CMJ height observed between 1 h and 3 h after the skeletal muscle-damaging exercise, there was no significant difference in CMJ height over time points (F=4.223, P=0.370) across the study. Diet type also had no significant effect on CMJ height (F=0.820, P=0.786) (Figure 3.2.).

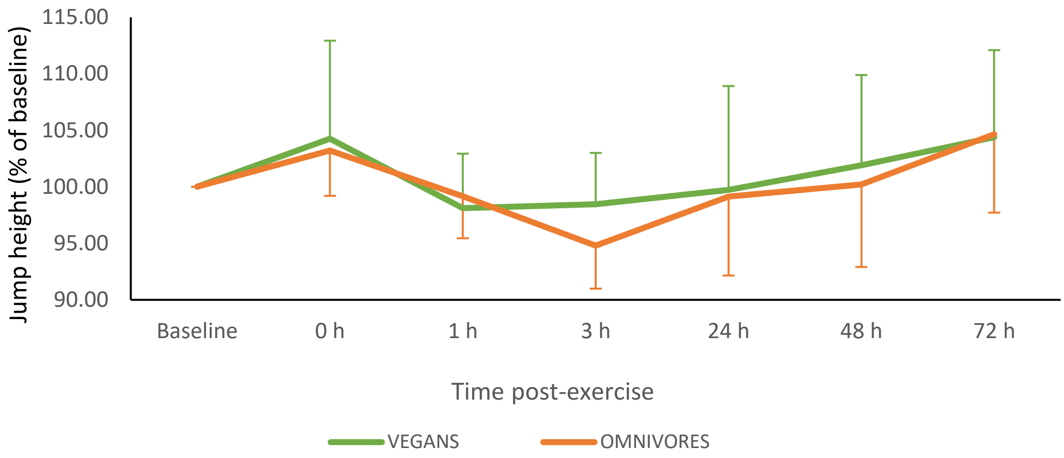

Figure 3.2. Change in CMJ height before and after the damaging exercise (% of baseline). Baseline values: Vegans 0.44±0.08 m, Omnivores 0.42±0.06 m. Values are mean ± SD. CMJ: countermovement jump.

3.4.2. Pain

There was a similar pattern of subjective pain at rest and during CMJs between vegans and omnivores, with an increase in pain that peaked at 24 h and returned to baseline at 72 h. There were significant differences observed over time points for generalised pain at rest (F=10.868, P=0.002) and during CMJ (F=8.428, P=0.003), (Figure 3.3.).

As above, subjective pain at specific muscles at rest was reaching a peak around 24 h after exercise and pain levels were mostly similar between vegans and omnivores. The difference in subjective pain at specific muscles at rest observed over time points was significant for the following muscles: (1) gastrocnemius, right leg (F=4.883, P=0.013) and left leg (F=4.510, P=0.014); (2) glutes, right leg (F=5.285, P=0.020); (3) hamstrings, right leg (F=3.828, P=0.042) and left leg (F=3.947, P=0.035); (4) inner thigh, right leg (F=5.110, P=0.020) and left leg (F=4.252, P=0.034); (5) outer thigh, right leg (F=10.279, P<0.001) and left leg (F=7.452, P=0.003) (Figures 3.3-3.8., respectively). The only muscles where a difference in pain was observed between groups, where vegans had less pain, were the glutes, left leg (F=12.377, P=0.017), (Figure 3.5.) and inner thigh, left leg (F=10.741, P=0.022), (Figure 3.7.).

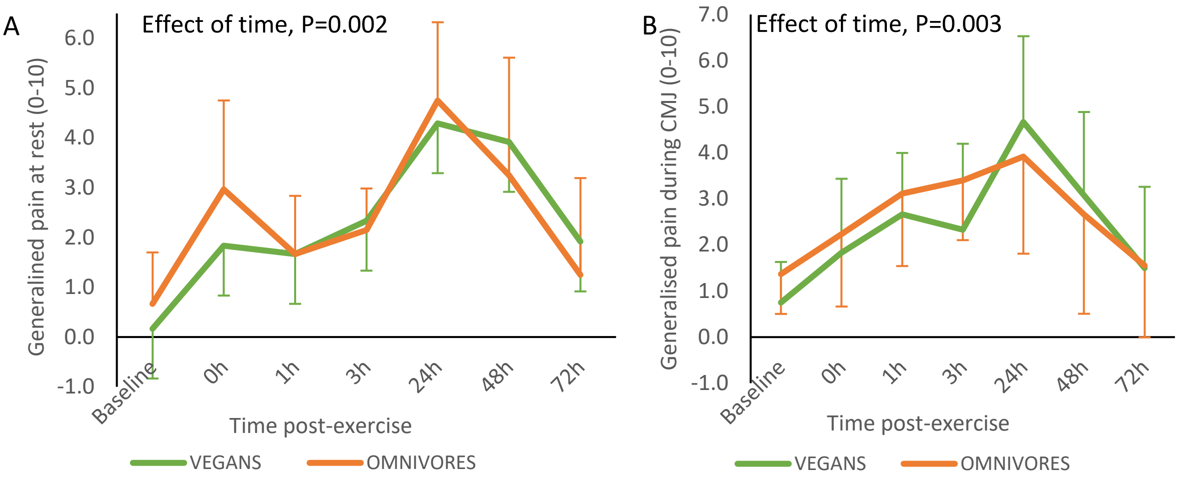

Figure 3.3. Subjective generalised pain scale (0-10), no pain (score 0) and worst pain (score 10). (A) at rest (F=10.868, P=0.002 for an effect of time using two-way repeated-measures ANOVA). (B) during CMJ (F=8.428, P=0.003 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD. CMJ: countermovement jump.

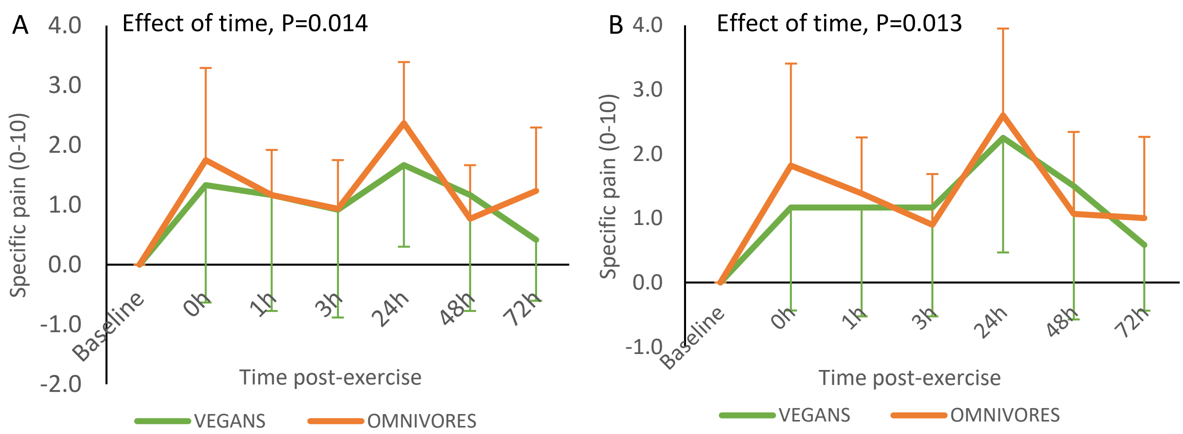

Figure 3.4. Subjective pain scale of the gastrocnemius muscle at rest (0-10), no pain (score 0) and worst pain (score 10). (A) left leg (F=4.510, P=0.014 for an effect of time using two-way repeated-measures ANOVA). (B) right leg (F=4.883, P=0.013 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD.

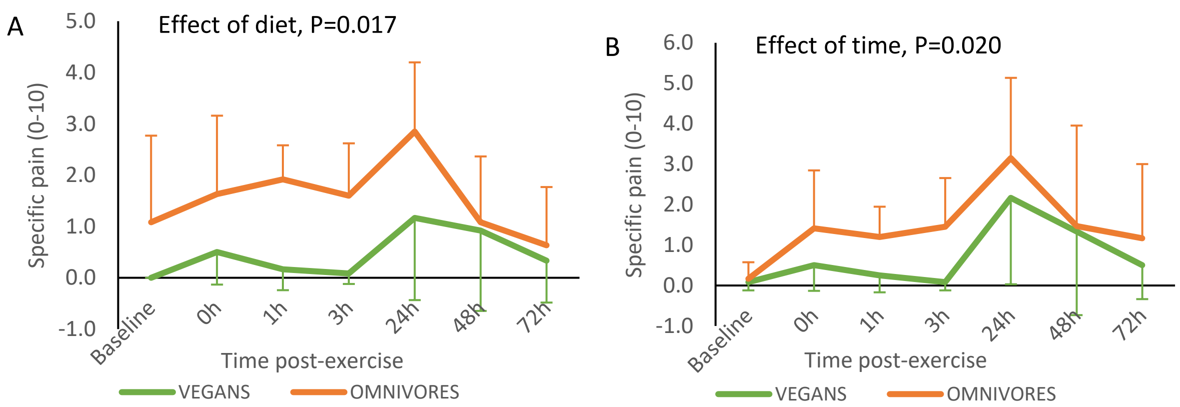

Figure 3.5. Subjective pain scale of the gluteus maximus muscle at rest (0-10), no pain (score 0) and worst pain (score 10). (A) left leg (F=12.377, P=0.017 for an effect of diet using two-way repeated-

measures ANOVA). (B) right leg (F=5.285, P=0.020 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD.

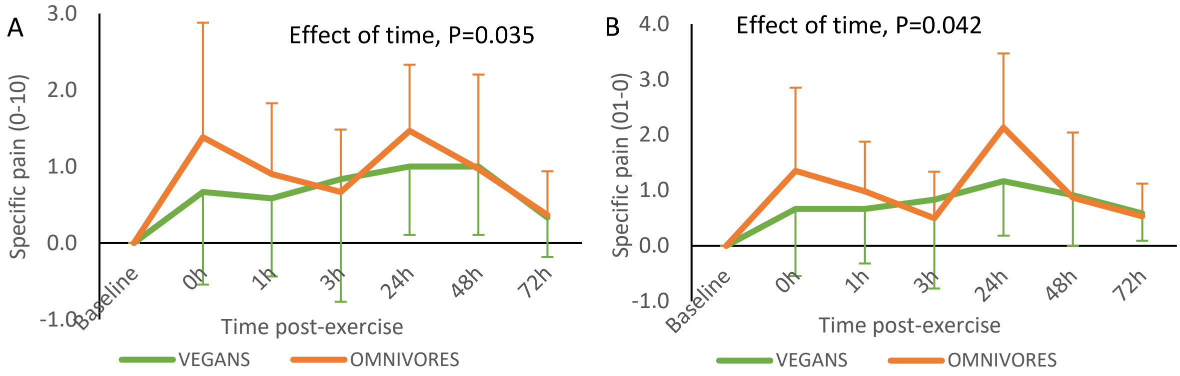

Figure 3.6. Subjective pain scale of the hamstrings muscle at rest (0-10), no pain (score 0) and worst pain (score 10). (A) left leg (F=3.947, P=0.035 for an effect of time using two-way repeated-measures ANOVA). (B) right leg (F=3.828, P=0.042 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD.

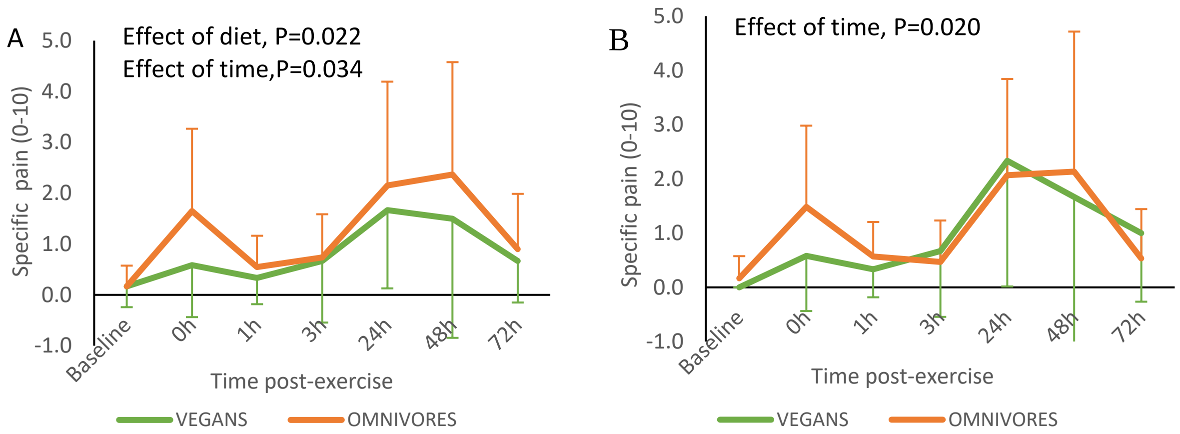

Figure 3.7. Subjective pain scale of the inner thigh muscle at rest (0-10), no pain (score 0) and worst pain (score 10). (A) left leg (F=10.741, P=0.022 for an effect of diet and F=4.252, P=0.034 for an effect of time using two-way repeated-measures ANOVA). (B) right leg (F=5.110, P=0.020 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD.

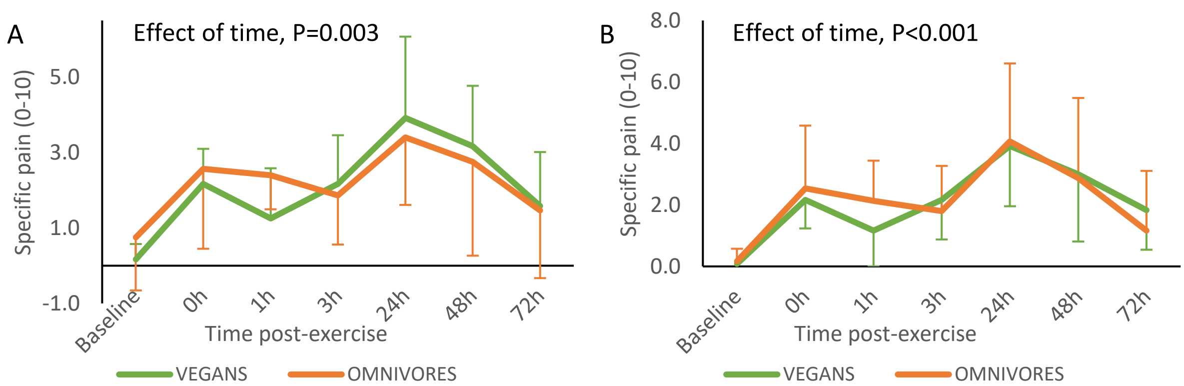

Figure 3.8. Subjective pain scale of the outer thigh muscle at rest (0-10), no pain (score 0) and worst pain (score 10). (A) left leg (F=7.452, P=0.003 for an effect of time using two-way repeated-measures

ANOVA). (B) right leg (F=10.279, P<0.001 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD

3.4.3. Pressure Pain Threshold (PPT)

PPT was not affected by the damaging exercise and was mostly similar between vegans and omnivores (Figures 3.9.-3.13.), except for calf lateral head, left leg, where omnivores had a slightly lower PPT (F=10.988, P=0.021), (Figure 3.14.). There was a tendency towards significance for the diet to impact the pain threshold, which was increased for vegans *vs.* omnivores in some muscles, including calf medial head, right leg (F=4.572, P=0.085) (Figure 3.15.) and vastus medialis right leg (F=6.387, P=0.053) (Figure 3.11.B). The Bonferroni post-hoc test only showed significant differences for calf medial head right leg, in which PPT measurements were higher in vegans at baseline, 0 hours, and 3 hours post-exercise (P=0.035, P=0.004, P=0.020), respectively. There were no significant differences over time for most of the muscles assessed, except for vastus medialis, right leg (F=3.455, P=0.043) (Figure 3.11.) and calf lateral head, right leg (F=3.480, P=0.050) (Figure 3.14.).

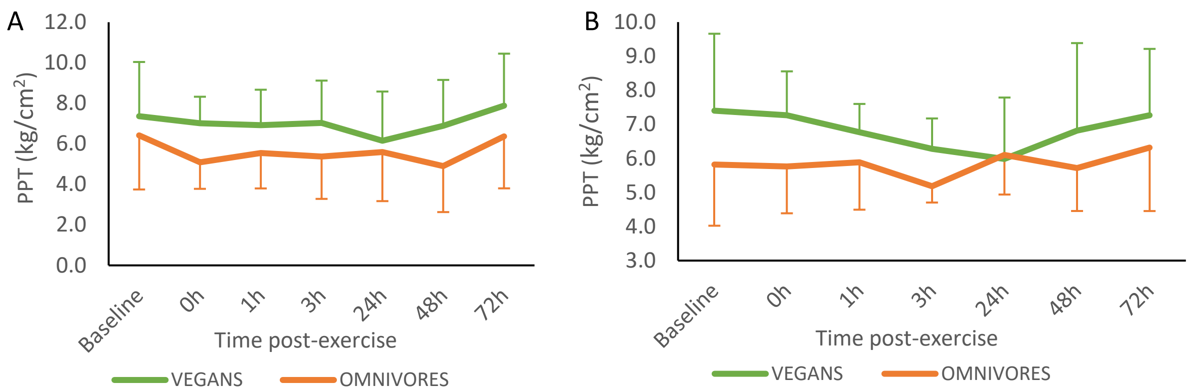

Figure 3.9. Pressure Pain Threshold (PPT) (kg/cm^2) of the vastus lateralis muscle at rest. (A) left leg. (B) right leg. Values are mean ± SD.

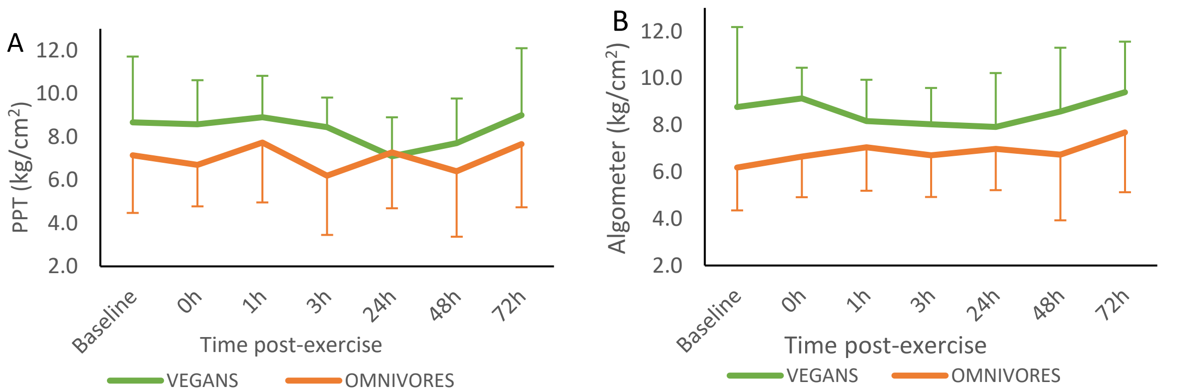

Figure 3.10. Pressure Pain Threshold (PPT) (kg/cm^2) of the rectus femoris muscle at rest. (A) left leg. (B) right leg. Values are mean ± SD.

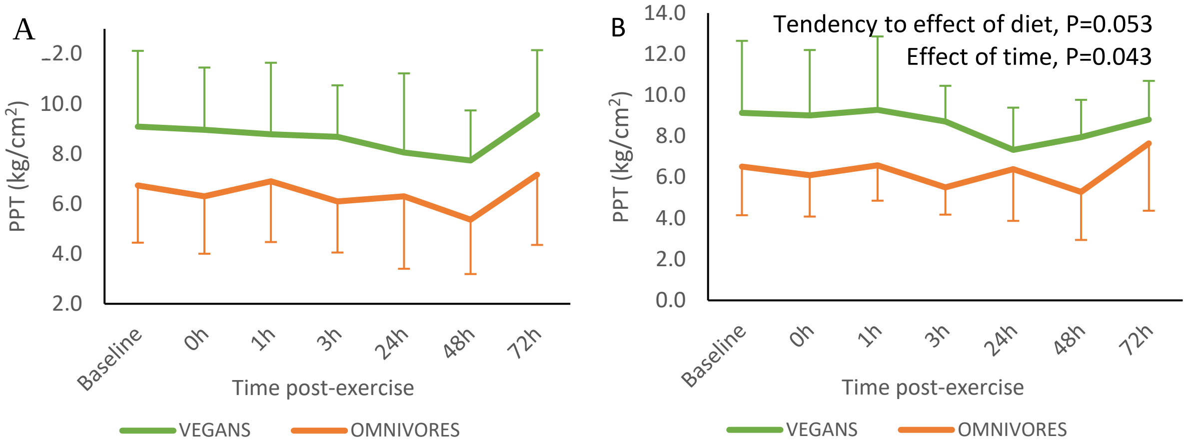

Figure 3.11. Pressure Pain Threshold (PPT) (kg/cm²) of the vastus medialis muscle at rest. (A) left leg. (B) right leg (F=6.387, P=0.053 for an effect of diet and F=3.455, P=0.043 for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD.

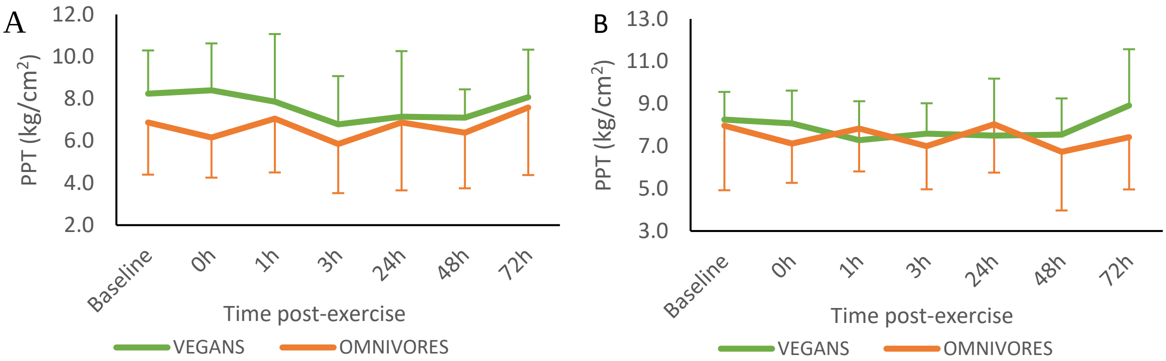

Figure 3.12. Pressure Pain Threshold (PPT) (kg/cm²) of the biceps femoris muscle at rest. (A) left leg. (B) right leg. Values are mean ± SD.

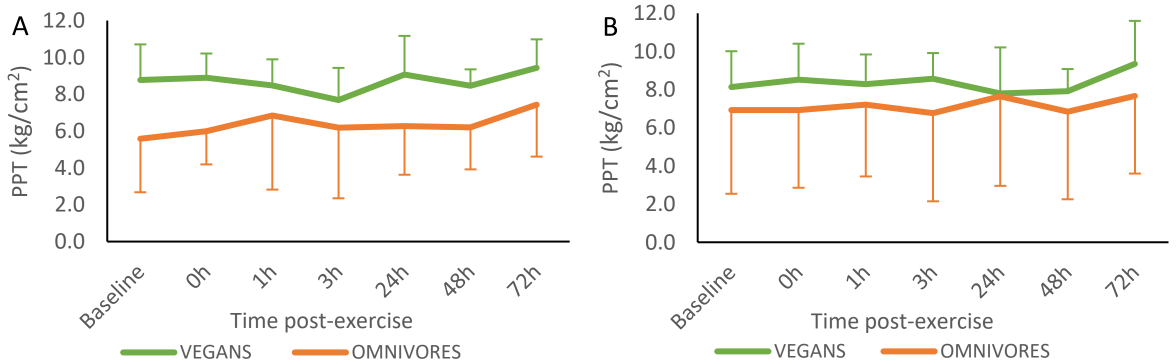

Figure 3.13. Pressure Pain Threshold (PPT) (kg/cm²) of the semitendinosus muscle at rest. (A) left leg. (B) right leg. Values are mean ± SD.

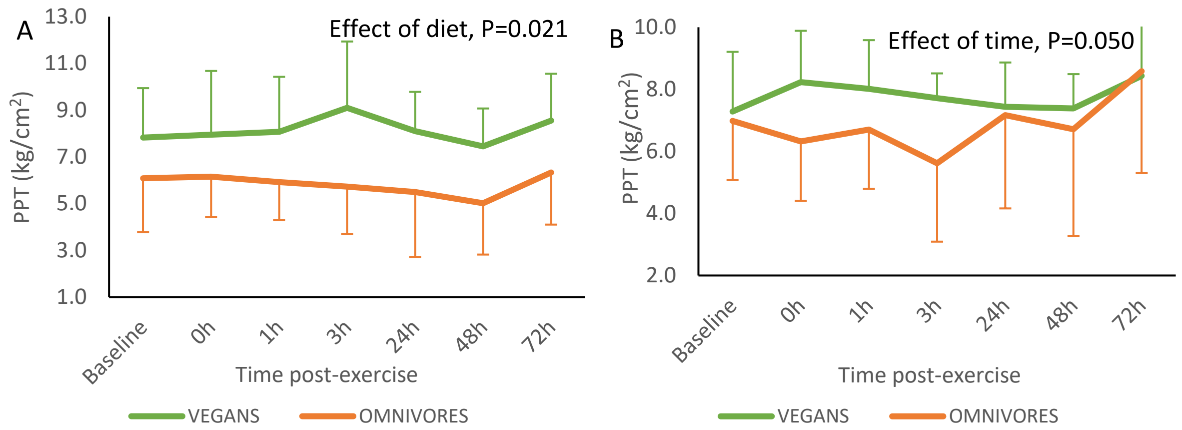

Figure 3.14. Pressure Pain Threshold (PPT) (kg/cm^2) of the calf lateral head muscle at rest. (A) left leg ($F=10.988$, $P=0.021$ for an effect of diet using two-way repeated-measures ANOVA). (B) right leg ($F=3.480$, $P=0.050$ for an effect of time using two-way repeated-measures ANOVA). Values are mean ± SD.

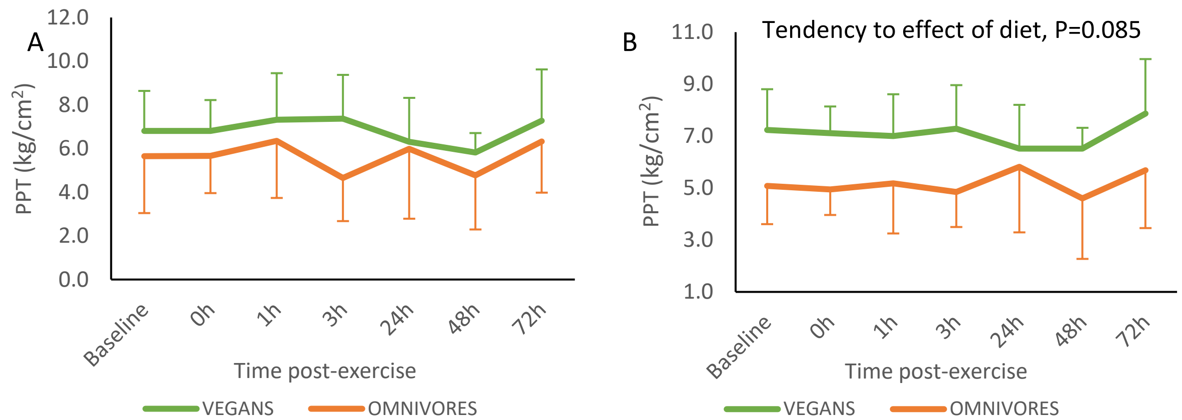

Figure 3.15. Pressure Pain Threshold (PPT) (kg/cm^2) of the calf medial head muscle at rest. (A) left leg. (B) right leg ($F=4.572$, $P=0.085$ for an effect of diet using two-way repeated-measures ANOVA). Values are mean ± SD.

3.5. Discussion

3.5.1. Skeletal muscle-damaging protocol

It appears that the skeletal muscle-damaging protocol (100 drop jumps) did not create the expected loss of force and performance expressed through CMJ height. These findings disagree with previous studies that have demonstrated that the protocol promotes a high level of exercise-induced muscle damage, expressed as a significant decrease in CMJ height, and subsequent soreness (Clifford et al., 2016, Chatzinikolaou et al., 2010, Kirby et al., 2012). In a study by Clifford et al., 2016, 30 recreationally active healthy males performed 100 drop jumps followed by beetroot juice supplementation protocol with different dosage regimes. Among the other parameters, CMJ and PPT threshold with an algometer were measured at pre-exercise, post-exercise, 24 h, 48 h and 72 h after the exercise. This study demonstrated a significant reduction of PPT (around 20%, $p<0.001$) and CMJ (between 6.5-9.7%), indicating that the protocol was effective in creating muscle damage (Clifford et al., 2016). In a study by Chatzinikolaou et al., 2010, 24 healthy males performed 50 jumps over 50 cm hurdles followed by 50 drop jumps. CMJ and DOMS were measured together with other parameters at pre-exercise, post-exercise, 24 h, 48 h, 96 h, and 120 h after the exercise. CMJ declined by 8-20% and there was a marked rise in DOMS (Chatzinikolaou et al., 2010). In a study by Kirby et al., 2012, 27 untrained males performed 100 drop jumps followed by leucine supplementation. CMJ, soreness (VAS) and other parameters were measured pre-exercise and at 24 h, 48 h, 72 h and 96 h post-exercise. CMJ significantly decreased from pre-exercise at 24 and 48 h, and muscle soreness increased across all time points (Kirby et al., 2012).

Though the exercise protocol did not produce a decrease in the height of CMJ, it produced significant skeletal muscle damage, as demonstrated by an increase in CK, rising by 1 hr post-exercise and peaking at around 24 h (100.37% and 104.3% increase from baseline, for vegans and omnivores, respectively) (Appendix A.2.). It appears that the exercise protocol created significant muscle damage but was not sufficient to create inflammation and subjective soreness, demonstrated by the absence of inflammatory cytokines in plasma (Appendix A.2.), nor the impact on subsequent muscle performance.

3.5.2. Pain

Skeletal muscle soreness peaked at 24 h after the exercise and this result is consistent with those of previous studies, which demonstrated that soreness is typically highest 24-48 h after strenuous exercise (Thompson et al., 1999, Kirby et al., 2012). A study by Thompson et al., 1999 demonstrated that, for 16 male university students, after 90 min of intermittent shuttle running and walking (Loughborough Intermittent Shuttle Test), the intensity of general and specific muscle soreness (VAS) was greater than baseline for 72 h after the test, peaking 24±48 h post-exercise. In a study by Kirby et al., 2012, 27 untrained males performed 100 drop jumps followed by leucine supplementation. Muscle soreness (VAS) significantly increased following exercise at 24, 48, 72 and 96 h.

The increase in pain was similar between rest and during CMJ, but that was not enough to reduce CMJ, which demonstrates that pain did not affect jump performance. This is in agreement with other studies, which have found weak correlations between measurements of soreness and functional and histological measurements of muscle damage (Rodenburg et al., 1993, Thompson et al., 1999). A study by Rodenburg et al., 1993, showed a weak correlation (0.34-0.63) between DOMS (VAS) and other functional and biochemical measures (maximal isotonic force, plasma CK and plasma myoglobin), for 27 male subjects during 5 days of recovery from 120 maximal eccentric contractions of the forearm flexors. Similarly, Thompson et al., 1999 demonstrated a weak correlation between muscle soreness (VAS) and measurements of blood CK (r= -0.36).

We have found differences in muscle soreness during recovery, with vegans having a higher pain threshold and lower subjective pain in specific muscle groups. However, those differences were inconsistent and sporadic. Other authors have already described external factors that could affect pain threshold. Elite and high-level athletes had increased pain tolerance, and higher heat pain thresholds, and reported lower pain intensity to thermal stimulation (Pettersen et al., 2020). There are also several studies that found that a vegan diet can reduce chronic pain in some conditions, including fibromyalgia and rheumatic disorders (Hänninen et al., 2000, Kaartinen et al., 2000, Barnard et al., 2019). In the study by Hänninen et al., 2000, 20 volunteers with fibromyalgia followed a vegan diet for 3 months and demonstrated significant improvement in the reduction of pain measured through VAS and stiffness, attributed to consumption of antioxidants and reduction of inflammatory environment. In the study by Kaartinen et al., 2000, 18 fibromalgia patients had a vegan diet for 3 months and reported a significant improvement in VAS. Therefore, it is possible that diet could affect muscle soreness, but the mechanisms linking habitual diet and DOMS are still unclear, and results of our study should be confirmed by larger or more powerful studies, as well as studies directed to address this specific question.

3.5.3. Pressure Pain Threshold (PPT)

The exercise protocol created pain, but this did not result in increased sensitivity through PPT measurements. These findings disagree with previous research that noted a decrease in PPT following a single bout of strenuous eccentric exercise (Bowtell et al., 2011, Clifford et al., 2016). The study by Bowtell et al., 2011, demonstrated a significant reduction in PPT after 24 and 48 h of recovery when ten well-trained male athletes completed two trials of 10 sets of 10 single-leg knee extensions at 80% one-repetition maximum. A study by Clifford et al., 2016, found that the PPT for 3 muscle groups was reduced at 24 h, 48 h and 72 h, after 100 drop jumps performed by 30 recreationally active healthy males.

At the same time, studies found that PPT has a different time course compared to VAS: it decreases the most one day after the exercise and remains the same until the end of recovery (Lau and Nosaka, 2011, Lau et al., 2013a). Soreness is often assessed using PPT in combination with VAS, as they assess two different aspects of pain (Lau et al., 2013b). Therefore, it is possible that the exercise protocol in the current study created significant soreness, but this was not expressed through increased sensitivity and lower PPT. Despite a clear increase in muscle soreness which was accompanied by muscle damage, the pain was not a factor that affected jump performance.

3.5.4. Countermovement jump (CMJ) height

Even though there was a slight decrease in CMJ height observed between 1 h and 3 h after the skeletal muscle-damaging exercise, there was no significant difference in CMJ height over time points across the study, and diet type also had no significant effect on CMJ height. Therefore, it appears that the muscle pain generated by the exercise protocol was not strong enough to affect the performance of drop jumps during the recovery period.

Even though CMJ is correlated to leg strength, one of the limiting factors of CMJ is the variation of performance related to jump technique. CMJ kinematic parameters, including greater ankle plantar flexion and shoulder flexion at take-off, were the factors explaining 58% variations in CMJ performance (McErlain-Naylor et al., 2014). One study has found that increasing the joint range of motion from 70% to 90% resulted in 17% of CMJ height (Moran and Wallace, 2007). A study by Domire and Challis, 2007 demonstrated that the depth of the squat can improve the jump performance due to an increase in time to develop joint torque (Domire and Challis, 2007). To attenuate that intrinsic variability, shoulder flexion at take-off was prevented by ensuring participants kept their hands on hips throughout the CMJ.

3.5.5. Dietary analysis

The diet of the vegan group had a higher energy intake compared to the omnivore group. (Appendix A.1.) This is not in agreement with previous studies, where vegan diets were characterised by lower energy content (Bakaloudi et al., 2021, Rizzo et al., 2013). This could be partly attributed to the higher variations in caloric content and other macronutrients within the vegan group. The analysis of participants' dietary information has revealed that the vegan group had a higher average consumption of carbohydrates, sugars and fibres compared with the omnivore group (Appendix A.1.), but the difference was not significant.

Higher consumption of carbohydrates by vegans is in agreement with the literature (Bakaloudi et al., 2021). The key body fuel stores determining exercise performance are skeletal muscle and liver glycogen (Coggan and Coyle, 1987). The time required to recover the exercise performance is impacted by the rate of glycogen repletion after the exercise (Koopman et al., 2007). Rates of glycogen resynthesis are dependent on the intake of dietary carbohydrates (Williams et al., 2003). Consuming high carbohydrate diet before the exercise can aid to offset exercise-induced changes in cytokines, and immune cells concentration and function (Walsh et al., 2011). Therefore, maintaining high carbohydrate intake during the period of training can provide benefits to exercise performance and aid the recovery (John, 2004).

This was further investigated and challenged by other authors (Miles et al., 2007, Depner et al., 2010, Green et al., 2008). Miles et al., 2007 have reported no difference in inflammation markers (IL-6, CRP and CK) and skeletal muscle damage responses (maximum force production and muscle soreness) from carbohydrate supplementation during 2 days of recovery from high-force elbow-flexion eccentric exercise. Depner et al., 2010 have compared muscle recovery (muscle soreness and strength loss) and inflammation markers (IL-1β, IL-6, CRP) from maximal high-force eccentric exercise between two groups with high- and low-carbohydrate supplementation. Perceived soreness was elevated at all time points post-exercise in both groups and was higher in the high compared to the low-carbohydrate group. Inflammation markers were also higher in the high-carbohydrate group. Green et al., 2008 found no difference in recovery (isometric quadriceps strength, lower extremity muscle soreness, CK) in female participants groups with post-exercise supplementation with carbohydrate, carbohydrate-protein and placebo drinks after eccentric exercise. Therefore, the differences in carbohydrate intake between vegans and omnivores, especially considering the large variability of habitual diets, may not be a key factor affecting muscle recovery in our study.

The vegan group diet was only slightly lower in protein compared to the omnivore group diet. This is not in agreement with other studies, where commonly vegan diets are lower in protein, primarily due to the exclusion of animal sources of protein (Bakaloudi et al., 2021, Rizzo et al., 2013). Consuming dietary protein assists in increasing the rate of skeletal muscle protein synthesis following muscle-damaging exercise, which is an important factor determining the rate of renewal of damaged muscle proteins, especially if the protein is consumed straight after the exercise (Phillips and Van Loon, 2011). Phillips and Van Loon, 2011 found that protein ingestion after exercise reduces indices of muscle damage, including creatine kinase. Though, how dietary protein ingestion might affect muscle damage and pain is still unknown.

An additional consideration is the nutritional quality of the dietary protein consumed. One of the potential consequences of a lack of animal-based protein in the diet is the lack of vitamin B_{12}. Average dietary intake of B_{12}, which was found to be highly correlated with the serum vitamin B_{12} concentration (Elorinne et al., 2016), was lower in the vegan group than the current recommendations of the Ministry of Health (MOH), New Zealand of 2.4 ug per day. Lack of vitamin B_{12} in the diet may potentially result in the reduced production of red blood cells, leukocytes and platelets (Anitha et al., 2014). This can have certain potential effect on skeletal muscle recovery, and future studies involving analysis of inflammatory markers could provide more information on the matter. The type of protein consumed also impacts the amount of cholesterol, as animal-based protein is one of the main dietary sources.

The omnivore group was consuming significantly more cholesterol than vegan group in their diets. Excessive dietary cholesterol consumption is associated with an increased risk of cardiovascular disease and in the long term can have an impact on cardiovascular health (Grundy, 2016). There are not many research studies that has analysed impact of dietary cholesterol on skeletal muscle recovery. One study has found an association between dietary cholesterol and increased soreness and loss of strength after exercise (Riechman et al., 2009). Further research is required to determine whether higher consumption of dietary cholesterol may contribute to higher pain thresholds in the omnivore group as demonstrated in this study.

The vegan group diets had more total fat compared to omnivore group diet, which is different to previous studies, where omnivorous diets were higher in fat (Pohl et al., 2021, Rizzo et al., 2013). However, those differences may be attributed to higher consumption of poly and mono-unsaturated fats by the vegans. Anti-inflammatory properties of polyunsaturated fats (PUFAs), including n-3 PUFA supplementation, are proposed to attenuate muscle subjective soreness measured by VAS and palpation after eccentric exercise (Jouris et al., 2011).

In line with the current literature, the vegan group diets were lower than recommended in zinc and iodine, while the omnivore group diets were lower than recommended in vitamin A equivalents, potassium, calcium and zinc (Bakaloudi et al., 2021, Elorinne et al., 2016). Consumption of vitamin C was similar in both groups, and consumption of vitamin E was higher in vegan groups, though consumption in the omnivore group was within the recommended levels. Vitamins C and E have antioxidant properties, protecting the body against consequences of oxidative stress, especially after the exercise shown to increase the oxidative stress (Powers and Lennon, 1999). There is an indication in the literature that a vegan diet can aim recovery through the reduction of oxidative stress, though we didn’t have significant differences in consumption of these vitamins between vegan and omnivore groups.

An insufficient dietary intake of iodine leads to insufficient production of thyroid hormones, which affects protein synthesis and could impact skeletal muscle functions (Kapil, 2007). Iodine is primarily lost through sweat, and high levels of sweating during exercise can deplete iodine levels, if the levels are not replaced through dietary intake. Sustained iodine loss through training combined with low dietary intake may have implications for thyroid status and possible consequences for athletic performance and recovery through increased fatigue (Smyth and Duntas, 2005).

Zinc has been found to affect skeletal muscle cell activation, proliferation and differentiation, and overall muscle regeneration (Hernández-Camacho et al., 2020). After an immediate increase following exercise, serum zinc levels decrease significantly during exercise recovery compared to pre-exercise levels (Chu et al., 2017). There is limited research available on the impact of zinc for skeletal muscle recovery. Based on the available evidence, zinc deficiency in athletes could have consequences for metabolic adaptations after the exercise and could have a negative effect on sport performance and recovery, with further research required to confirm these findings (Hernández-Camacho et al., 2020).

The vegan group had significantly higher consumption of riboflavin and water. Though the omnivore group consumed significantly less riboflavin in their diet, their dietary intake was still within the recommended ranges. Water intake in the omnivore group was below the recommended 2,600 ml. Results from studies indicate that dehydration negatively impacts muscular strength, power, and endurance, and rehydration is beneficial for skeletal muscle recovery (Judelson et al., 2007, Harris et al., 2019). However, that broad recommendation of water requirement is being challenged by some authors (Yamada et al., 2022).

Intake of micronutrients below requirements does not always result in vitamin deficiencies. Though vitamin deficiencies should not be disregarded, it is not always associated with health impairments (Bakaloudi et al., 2021) and has a potential low immediate impact on skeletal muscle recovery after exercise.

3.6. Conclusion

Our study has found no differences in skeletal muscle recovery and soreness between omnivores and vegans after a single bout of eccentric exercise. Mechanisms linking habitual diet and DOMS are still unclear, and results of our study should be confirmed by larger or more powerful studies.

CHAPTER 4.
CONCLUSION AND RECOMMENDATIONS

The study has achieved its aim to compare the recovery of skeletal muscle function post-strenuous exercise between healthy young males on a vegan diet with those on an omnivorous diet. The study found no significant differences in the recovery of muscle function following a single bout of muscle-damaging exercise between healthy young males on an omnivorous diet or a vegan diet.

4.1. Research impact

Skeletal muscle recovery is an important part of any exercise and fitness programme for athletes and any person with an active lifestyle. Adequate recovery is necessary for athletes to be able to perform to their maximum potential and the lack of adequate recovery can be an obstacle in competitive performance. Therefore, full recovery is vital for athletic performance (Barnett, 2006) and is important for the general population to maintain an active lifestyle. Optimizing skeletal muscle recovery can provide athletes and the general population with a competitive advantage and can reduce the time between training sessions, and nutrition is one of the important relevant factors under investigation in the literature. As plant-based diets become more popular, it is very important to understand what impact consumption of plant-based diets will have on muscle recovery.

The results of the research provide initial data that can be used to base further studies to address specific issues on the variety of omnivorous and vegan diets, types and intensity of exercise protocols and pain assessments.

4.2. Study strengths and limitations

The main study strengths were that the study was completed within a limited timeframe and budget. Researchers have independently completed all stages of participant recruitment and data collection, with a 100% rate of participants retention.

One of the main study limitations is the small sample size, with only young males as research subjects. Though one of the study selection criteria was participants with recreational levels of fitness, we have noticed that the fitness level of participants varied, which had an impact on the ability of the protocol to create detectable skeletal muscle damage.

It appears that CMJ depends on some factors, including jump technique, and is not the most precise method of measurement of force and performance. The study has some limitations in budget and equipment, which resulted in the avoidance of using skeletal muscle biopsies and magnetic resonance imaging, which are considered the most reliable direct measures.

Though a habitual diet is ecologically valid and closer to real life, a study based on the habitual diet of the participant is subject to significant variations in the intake of macro and micronutrients, rather than a standardized diet with controlled caloric and nutritional intake.

Another study limitation is the presence of other factors that have an impact on skeletal muscle recovery, including individual variations in response (see section 2.3.) which could not be accounted for due to technical constraints.

4.3. Future perspectives

Further studies with a larger sample size including both male and female participants would be required to account for a variety of hormonal status, age, fitness, and socioeconomic levels and would reduce the risk of having inhomogeneous or biased groups.

Applying an exercise protocol individualized to achieve maximum effort in future studies would help to achieve detectable exercise-induced damage, detected through a decreased force and performance after the exercise. Future studies should include fitness tests for participants to achieve homogeneity in the fitness level of participants.

As CMJ depends on many factors, more precise methods of measurement of force and performance can be used, including dynamometers and strain gauges.

Applying a standardized diet, with controlled caloric and nutritional intake focusing on the animal or plant-based source of protein, as opposed to the habitual diet of the participant, would help to control the homogeneity of the dietary intervention. Having a controlled diet after exercise would also help to eliminate the impact of diet on recovery.

Our study has found no differences in skeletal muscle recovery between omnivores and vegans after a single bout of an eccentric, muscle-damaging exercise. A few sporadic results showed that vegans have experienced less muscle soreness during the recovery period, however, it should be confirmed by larger or more powerful studies, as well as studies directed to address this specific question.

APPENDICES

Appendix A. Supplementary results

Appendix A.1. Dietary analysis

Diet records were entered into Foodworks Professional diet analysis program (Version 10, 2020, Xyris Software) and reports were obtained containing micro- and macro-nutrients breakdowns. For each micro- and macro-nutrient group average, standard deviation and P-values were calculated for both vegan and omnivore groups.

Table A.1. Nutritional analysis of 3-day food diaries. N=6 omnivores, N=5 vegans.

Nutrient	Recommended intake by Ministry of Health (MOH)	Vegan (Mean± SD)	Omnivores (Mean± SD)	P-value
Energy (kJ)		12584.3± 5,577.7	10,081± 1,733.0	0.382
Protein (g)	0.8-1g/kg	102.5± 37.0	108.3± 49.9	0.830
Total Fat (g)	20-35% EER	134.2± 59.2	84.3± 20.9	0.135
Sat fat (g)	<10% EER	33.3± 20.5	25.3± 7.4	0.445
Trans fat (g)		0.4± 0.4	1.1± 0.6	*0.046
Poly fat (g)		32.8± 22.2	16.7± 9.3	0.187
Mono fat (g)		48.7± 26.8	31.2± 8.2	0.224
Cholesterol (mg)		5.3± 3.8	289.0± 174.2	*0.010
Carbohydrate (g)	45-65% EER	321.1± 172.2	287.8± 103.4	0.717
Sugars total (g)		111.1± 36.0	85.7± 74.2	0.482
Starch (g)		204.5± 138.1	192.8± 71.4	0.871
Water (ml)	2600 ml/d	2,717.7± 696.0	1,643.9± 766.9	*0.038
Fibre (g)	30 g/d	56.7± 25.8	29.3± 11.0	0.075
Thiamin (mg)	1.2 mg/day	1.9± 0.5	1.5± 0.9	0.361
Riboflavin (mg)	1.3 mg/day	2.0± 0.3	1.4± 0.6	*0.048
Niacin (mg)		17.3± 6.1	22.4± 11.9	0.384
Niacin equivalents (mg)	16 mg/day	36.7± 13.4	43.0± 20.9	0.556

Nutrient	Recommended intake by Ministry of Health (MOH)	Vegan (Mean± SD)	Omnivores (Mean± SD)	P-value
Vit C (mg)	45 mg/day	127.2± 70.9	137.6± 106.0	0.851
Vit E (mg)	10 mg/day	43.7± 47.7	15.4± 7.5	0.258
Tocopherol, alpha (mg)		24.2± 16.7	12.6± 5.9	0.202
Vit B_6 (mg)	1.3 mg/day	1.7± 0.6	1.4± 0.8	0.396
Vit B_{12} (ug)	2.4 µg/day	2.1± 0.9	112.7± 268.5	0.359
Folate total (ug)	400 µg/day	624.0± 273.6	575.5± 518.0	0.848
Folic acid (ug)		81.5± 95.3	90.9± 70.6	0.860
Food folate (ug)		542.6± 218.3	479.7± 464.9	0.776
Vit A equivalents (ug)	900 µg/day	1,070.5± 387.2	730.6± 468.4	0.220
Retinol (ug)		171.6± 108.4	187.8± 86.1	0.793
Beta Carotene equivalents (ug)		5,389.7± 2,199.4	3,256.9± 2,745.0	0.186
Beta Carotene (ug)		4,200.3± 1,850.7	2,700.1± 2,307.3	0.262
Sodium (mg)	2,000 mg/day	3,461.9± 836.4	2,168.7± 1,213.6	0.068
Potassium (mg)	3,800 mg/day	4,104.6± 1,560.8	3,139.5± 1,417.6	0.317
Magnesium (mg)	420 mg/day	707.2± 352.6	361.7± 176.1	0.096
Calcium (mg)	1,000 mg/day	1,135.9± 539.6	625.7± 370.4	0.117
Phosphorus (mg)	1,000 mg/day	1,820.6± 817.4	1,498.9± 762.0	0.521
Iron (mg)	8 mg/day	19.2± 7.2	13.1± 6.1	0.168
Zinc (mg)	14 mg/day	13.2± 7.0	10.5± 4.4	0.473
Selenium (ug)	70 µg/day	177.1± 299.0	92.1± 33.5	0.561
Iodine (ug)	150 µg/day	128.8± 50.3	367.0± 625.1	0.395

* Significant difference calculated by unpaired two-tailed t-test.

Appendix A.2. Thesis "The impact of vegan vs. omnivorous diets on biomarkers of inflammation in muscle recovery" presented in partial fulfilment of the requirements for the degree of Master of Science Nutrition and Dietetics at Massey University, Auckland, New Zealand (Leach, 2023).

N.B. Measurements were performed simultaneously with our study, using the same participants and research design.

Creatine Kinase (CK) Results

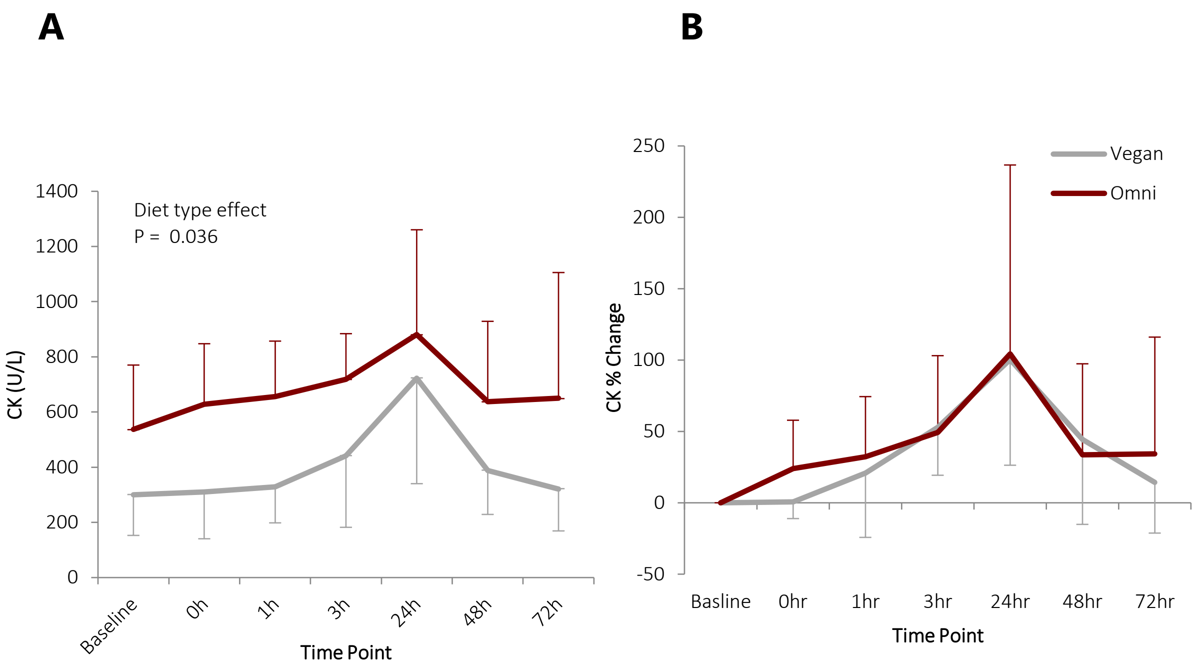

Figure A.2.1. Plasma CK values over the time course of muscle damage-inducing exercise. Panel A: Absolute Values: Diet type effect: F = 8.076, P = 0.036; Time effect: F = 2.124, P = 0.177; Interaction between diet and time: F = 0.206, P = 0.825. Panel B: % change from baseline: Diet type effect: F = 0.099, P = 0.766; Time effect: F = 3.451, P = 0.091; Interaction between diet and time: F = 0.355, P = 0.772. STATS method: two way within-subjects ANOVA, repeated measures. Values presented as mean ± SD. Vegan N = 6; Omnivore N = 6.

C-reactive protein (CRP)

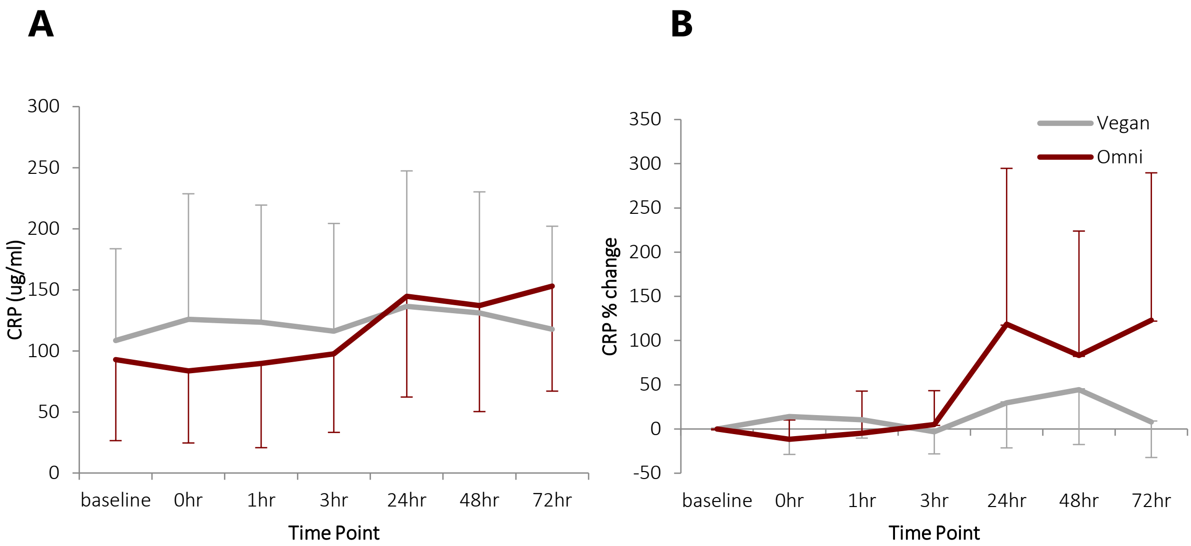

Figure A.2.2. Plasma CRP values over the time course of muscle damage-inducing exercise. Panel A: Absolute Values: Diet Type effect: F = 0.023, P = 0.886; Time effect: F = 2.913, P = 0.110; Interaction between diet and time: F = 2.452, P = 0.153. Panel B: % change from baseline: Diet type effect: F = 0.958, P = 0.373; Time effect: F = 3.607, P = 0.100; Interaction between diet and time: F = 2.452, P = 0.153. STATS method: two way within-subjects ANOVA, repeated measures. Values presented as mean ± SD. Vegan N = 6; Omnivore N = 6.

Tumor Necrosis Factor alpha (TNF-α)

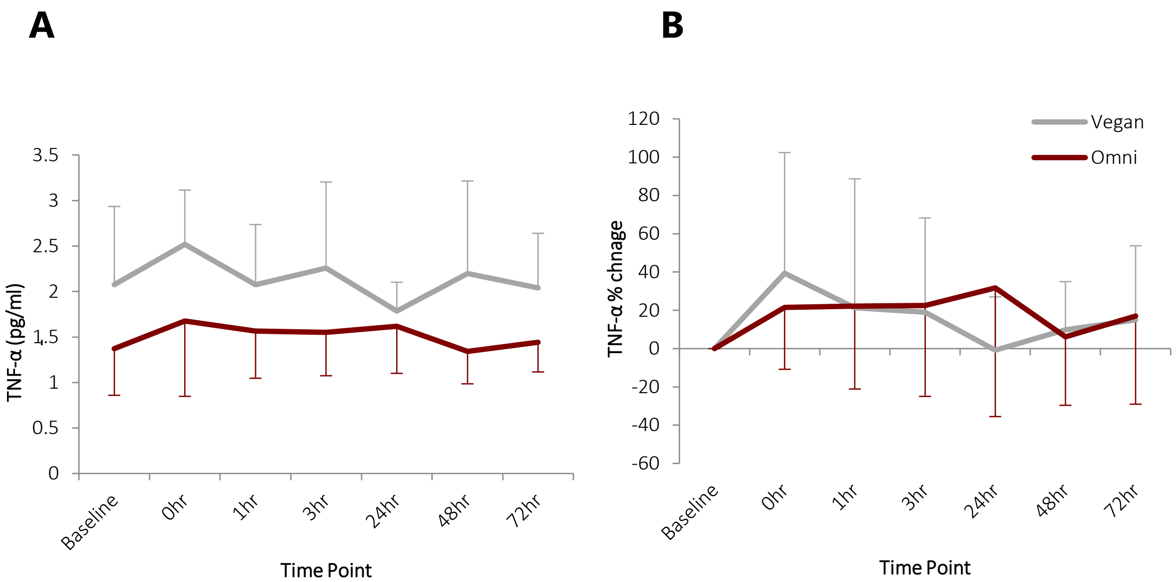

Figure A.2.3. Plasma TNF-α values values over the time course of muscle damage-inducing exercise. Panel A: Absolute Values: Diet type effect: F = 2.916, P = 0.149; Time effect: F = 1.106, P = 0.377;

Interaction between diet and time: F = 0.788, P = 0.473. Panel B: % change from baseline: Diet type effect: F = 0.050, P = 0.831; Time effect: F = 1.153, P = 0.358; Interaction between diet and time: F = 0.614, P = 0.579. STATS method: two way within-subjects ANOVA, repeated measures. Values presented as mean ± SD. Vegan N = 6; Omnivore N = 6.

Interleukin 1-beta (IL-1β)

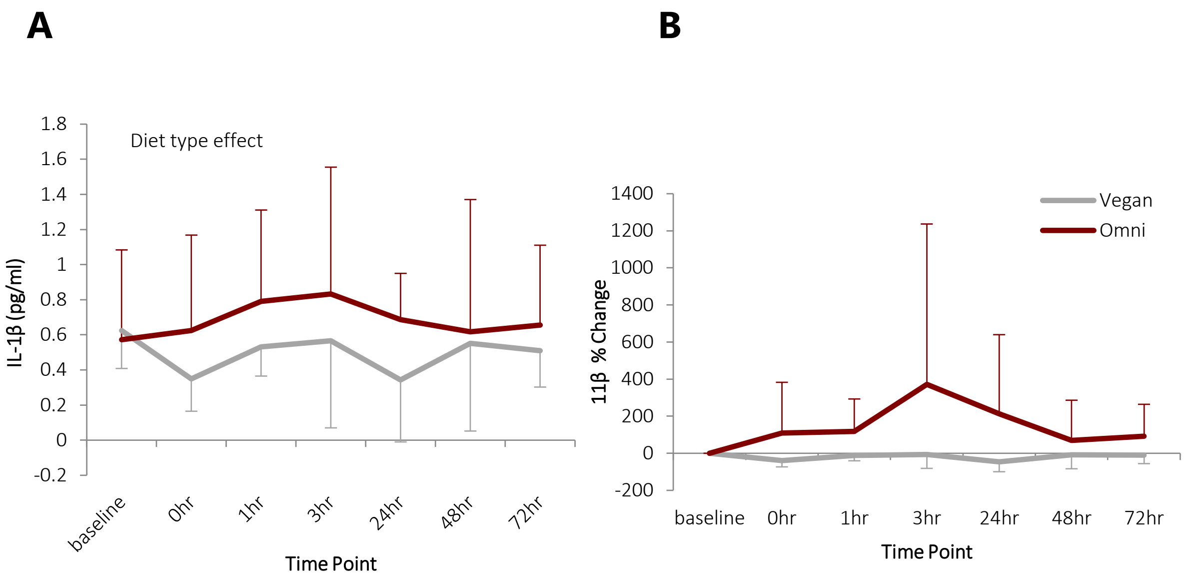

Figure A.2.4. Plasma IL-1β values over the time course of muscle damage-inducing exercise. Panel A: Absolute Values: Diet type effect: F = 1.01, P = 0.0341; Time effect: F = 0.58, P = 0.7412; Interaction between diet and time: F = 0.55, P = 0.7655. Panel B: % change from baseline: Diet type effect: F = 1.04, P = 0.3367; Time effect: F = 0.65, P = 0.6867; Interaction between diet and time: F = 0.73, P = 0.6277. STATS method: two way within-subjects ANOVA, repeated measures. Values presented as mean ± SD. Vegan N = 4; Omnivore N = 6.

Appendix B. Questionnaires and material used in conducting the research

Appendix B.1. Participant Information Sheet

PARTICIPANT INFORMATION SHEET
VEGAN VS OMNIVOROUS DIETS - IMPACT ON MUSCLE RECOVERY

Researchers: Anastasia Woolsey; Tam Leach

Introduction

We are Massey University students studying toward a MSc Nutrition and Dietetics, supervised by Associate Professor Andy Foskett and Dr Kaio Vitzel and we are recruiting men to participate in a project investigating recovery of muscle after exercise, in those consuming a vegan diet (diets absent of any animal product) 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, you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this. 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, as people move towards lifestyles deemed to be healthier and more environmentally friendly. Plant-based and vegan diets may support good health and prolong life expectancy, and may impact on sports performance. Recovery is an important consideration for anyone engaged in regular sports or exercise, since a speedy recovery prevents injury and gets you ready 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 vegan diet vs omnivorous diet on muscle recovery after exercise.

Who can take part? We are looking for men who:

- Have adhered to either a vegan or an omnivore diet for at least the past 2 years.
- Recreationally active
- 18-45 years of age
- Non-smoker
- No chronic health conditions

The study is not suitable for Individuals who are advanced/elite athletes (someone who trains four or more times per week for their sport and competes at a high level), or performs lower limb strength/resistance training or has any conditions identified on the “Health Screening Questionnaire”.

Project Procedures

The study requires you to attend five 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.

This will take around 45-60 minutes to complete.
Before you start the other tests, you will be asked to record the food that you eat for 3 days. The following 4 visits will comprise the Research Trials**.**

Day 1:
Baseline tests
We will measure your body mass and stature.

Participants will first perform baseline measures where we will ask you to perform 3 vertical jumps on a floor mat to measure how high you can jump. We will also ask you to tell us how sore your leg feels at various times, using a scale system and a piece of equipment that presses gently on your leg, called an algometer. We will then take some blood samples to measure blood markers associated with muscle inflammation.

These initial tests will take about 30-40min to complete.

Exercise regimen

Next, you will be asked to do an exercise regimen. For this we will ask you to perform 100 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 from the ground. There will be a 10 second

interval between jumps and 2 min rest between each set of 20 jumps and we will direct you to do this activity safely. This will take about 30 mins.

You will then repeat the baseline tests as previously outlined, which will also be done again after 1 hour and 3 hours and will take about 30 min each time.

Between tests you can rest, have lunch, watch television etc.

We will also ask you to complete a one-day food diary for the day of the study, and another 3- day food diary on days where you are eating normally.

Day 2, 3 and 4:

At 24 hours (day 2), 48 hours (day 3) and 72 hours (day 4) after the exercise regimen, you will return to the lab and repeat the baseline tests as above.

This will take about 30 min each time. That is the end of the tests.

Things you will need to avoid during the study

You will need to abstain from alcohol, caffeine and taking supplements for 24 hours before the test and for the duration of the tests You will also need to avoid doing any exercise for 48 h 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 from exercise. 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.

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 regimen. 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 procedure they are carrying out and only fully qualified phlebotomists will be taking blood samples. We will also have support staff available in case you do experience any adverse affects.

Will any costs be reimbursed?

Participants will be offered a $50 koha 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 identifiable information is held at Massy University (Auckland campus) during the study and stored for no longer than five (5) years, then destroyed. All storage will comply with local and/or international data security guidelines.

Who has approved the study?

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 22/10. If you have any concerns about the conduct of this research, please contact Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@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 as a result 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 k.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

Tam Leach
0212606840
MSc Nutrition and Dietetics Student t.leach@massey.ac.nz

Anastasia Woolsey
02108685556
MSc Nutrition and Dietetics Student a.vulsi@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 of 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 MoE and MoH.

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 22/10. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

Participant's Rights

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

- Decline to answer any particular 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 B.2.Health Screening Questionnaire

Vegan diet vs. Omnivorous diet:

Impact on recovery of muscle function

Health Screening Questionnaire

Name:____

Phone: _______________________________

Age: ________________

Gender:______________

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

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

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

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

Qu 7. Do you have epilepsy or have you ever had a seizure of any sort? Explain?

Yes ☐ No ☐

____

____

____

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

Yes ☐ No ☐

____

Qu 9. 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 10. 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 11. Have you recently undergone surgery or are you carrying an injury? Explain.

Yes ☐ No ☐

____

____

____

Qu 12. Do you smoke?

Yes ☐ No ☐

Qu 13. Do you perform regular leg resistance/strength exercise?

Yes ☐ No ☐

Qu 14. Are you currently ill in any way? Please explain.

Yes ☐ No ☐

____

____

____

Qu 15. Are you aware of any other reason why you should not participate in physical exercise without medical supervision? If so, what?

Yes ☐ No ☐

____

____

____

Qu 16. Do you have any issues with having your blood taken?

Yes ☐ No ☐

Qu 17. Do you have any bleeding or healing disorders?

Yes ☐ ☐ No

Qu 18. Are there any issues that may prevent you from completing an approximately 60 min of jumping of 0.6m box? If yes, please explain.

Yes ☐ ☐ No

____

____

____

Qu 19. Has your diet in the past 2 years included foods of animal origin (meat, chicken, fish, eggs, milk, dairy products)

Yes ☐ ☐ **No**

If YES, how many times per week do you consume animal-derived foods?

3 or more times per week ☐

Less than 3 times per week ☐

I have read, understood and completed this questionnaire.

Signature ***(Participant):*** ____ Date: ___________________

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Appendix B.3. Consent Letter

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 particular 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 _________**

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