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Nutritional intake, diet quality and exercise training: an exploration of adults following vegetarian-based dietary patterns

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Abstract

Vegetarian-based dietary patterns have been associated with protection against many chronic diseases. These benefits have been attributed to the high intakes of food components such as fruits, vegetables, nuts, seeds and legumes and absence of animal foods as part of a vegetarian diet, with some researchers suggesting there may be anti-inflammatory effects of following a vegetarian-based diet. Interestingly, there are growing numbers of individuals adopting a diet of this nature, with athletes being overrepresented in the consumption of vegetarian-based diets. Despite the increase in vegetarian-based diet popularity, there is a lack of research describing the motives, dietary behaviours, supplementation patterns, nutrient intakes and diet quality of individuals following a vegetarian-based diet, particularly for athletes. If differences in nutritional composition between athletes following omnivorous and vegetarian-based dietary patterns exist, there may also be various differences in exercise related physiological outcomes including inflammatory and immunological markers. The central hypothesis of this thesis is that due to differences in nutrient composition and diet quality between vegetarian and non-vegetarian-based dietary patterns, disparities will result in various inflammatory and immune biomarkers as well as other endurance exercise related physiology.

In order to explore the dietary behaviors, supplementation patterns and motives in this population group, an online survey was implemented (Study 1a). Here, it was found that self-reported recreational and competitive athletes adopting a vegetarian-based diet were likely doing so with the aspiration of improving their exercise performance. A nutrient analysis in a subset of this study (Study 1b) suggested that a vegetarian-based dietary pattern could provide a high diet quality for recreational athletes with sufficient nutrients to support

physical activity. However, intake of some nutrients in this self-reported vegetarian-based population were insufficient such as for vitamin B12 and long chain omega-3 polyunsaturated fatty acids. As this was an online, self-reported population of individuals, it was important to contrast dietary patterns between vegetarian and non-vegetarian dietary patterns and to explore if differences in inflammatory and immune markers as a function of dietary pattern existed between groups which also have relevance to exercise outcomes.

A systematic review and meta-analysis (Study 2) was performed to examine the relationship between vegetarian-based dietary patterns and inflammatory and immune markers in both observational and intervention studies. Those following vegetarian-based diets were found to have significantly lower concentrations of CRP, fibrinogen and total leukocytes compared to those following non-vegetarian dietary patterns in observational studies. Given markers of inflammation and immune function were disparate between those following a vegetarian compared to non-vegetarian diet, it was plausible that these differences might translate into modest reductions in the inflammatory response during endurance performance, however, limited research in athletes following vegetarian-based and non-vegetarian based diets existed in the literature.

A laboratory study (Study 3) was implemented to substantiate the findings from study 1b and contrast the nutritional composition and diet quality in a group of free-living vegan and non-vegan endurance trained athletes in a controlled setting. The vegan group consumed significantly less protein, total, saturated, monounsaturated and trans fats, and cholesterol whilst consuming more dietary fibre compared to the omnivorous group. As a percentage of total energy, the vegan group consumed significantly more energy from carbohydrates (49.3% vs 41.5%, $p = 0.0068$), significantly less energy from protein (19.0% vs 14.2%,

p<0.001) and less energy from fat (29.3% vs 33.8%. p = 0.0583). As intake of dietary LC n3 PUFA was observed to be low in the online survey, long chain poly unsaturated fatty acids were a focus of this study. The O3I in both groups was suboptimal yet the vegan group was critically low with a mean O3I of 4.13%; with some individuals <4% compared to a mean of 5.40% in the omnivorous group.

A laboratory study (Study 4) was also performed to build on the findings from Studies 1 to 3 by enabling exercise related physiological markers to be examined in a controlled setting. Males aged 18 to 55 years old, engaging in >4 hours training/week and following a vegan (greater than 6 months) or omnivorous dietary pattern were recruited, and a seven day food and training diary was collected to assess training volume and diet quality. An incremental ramp running protocol was used to determine peak aerobic power and exercise testing was during walking and clamped conditions of 60% and 90% of their maximum aerobic capacity was performed. Significantly lowered red blood cell counts, haemotocrit and haemoglobin were identified in the vegan dietary group which appeared to have influenced oxygen carrying capacity especially at peak aerobic power. Mean heart rate, heart rate variability and oxygen consumption were all comparable at each exercise intensity. Inflammatory and immune biomarkers were comparable between the dietary groups, although, a more prolonged exercise duration may have induced a more pronounced response required to observe potential differences. More research is required to substantiate the findings given the low numbers in this study.

The findings from this thesis in part support the central hypothesis, whereby those consuming vegetarian-based dietary patterns are likely to have differing nutrient intakes compared to those following non-vegetarian-based dietary patterns, which may favorably modulate

inflammatory and immunological responses in the general population. However, these differences in nutrient intakes did not translate into disparate inflammatory and immunological responses prior to, or post exercise in young, healthy trained male athletes following either a vegan or non-vegan dietary pattern. There may be differences in red blood cell counts and haemoglobin between athletes following vegetarian or non-vegetarian diets which is known to modulate oxygen carrying capacity. This was reflected in the current study where a significant increase in muscle oxygen desaturation at the point of exhaustion was observed in athletes following vegan, compared to non-vegan diets. Additional research is warranted to explore this relationship.

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Certification

I, Joel Craddock, declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Doctor of Philosophy, in the School of Medicine from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

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Joel Craddock

15th October 2021

List of Names or Abbreviations

α-LA: alpha-linolenic acid

AA: Arachidonic acid

AHEI-2010: Alternate Healthy Eating Index-2010

AI: Adequate Intake

APD: Accredited Practising Dietitian

ASA24-AU: Automated Self-Administered 24-hour-Australian

AUSNUT: Australian Food and Nutrient Database

BCAA: Branched Chain Amino Acids

BMI: Body mass index

CF3: Compliment factor 3

CHERRIES: Checklist for Reporting Results of Internet E-Surveys

CHO: Carbohydrate

CVI: Content Validity Index

CRP: C-reactive protein

CVD: cardiovascular disease

DFA α1: Detrended fluctuation analysis

DHA: Docosahexaenoic acid

DPA: Docosapentaenoic acid

ECG: Electrocardiograph

EAR: Estimated Average Requirement

ELISA: Enzyme-Linked Immunosorbent Assay

EPA: Eicosapentaenoic acid

FFQ: Food Frequency Questionnaire

HDL: High-density lipoprotein

HR: Heart rate

HRV: Heart rate variability

IgA: Immunoglobulin A

IgD: Immunoglobulin D

IgE: Immunoglobulin E

IgG: Immunoglobulin G

IL-1: Interleukin-1

IL-2: Interleukin

IL-6: Interleukin-6

IL-10: Interleukin-10

IPAQ-SF: The International and Physical Activity Questionnaire-Short Form

IQR: interquartile range

JC: Joel Craddock

LC n-3 PUFA: long-chain omega-3 polyunsaturated fatty acids

LOV: lacto-ovo-vegetarian

LV: Lacto-vegetarian

MET: Metabolic equivalent

NK: Natural Killer

O3I: Omega-3 Index

PBS: Phosphate buffered saline

DPI: Dietary Phytochemical Index

PRISMA: Preferred Reporting of Systematic Reviews and Meta-analyses

PROSPERO: Prospective Register of Systematic Reviews

RCTs: Randomised controlled trials

ROS: Reactive Oxygen Species

RT: Room temperature

SD1: Poincaré plot standard deviation perpendicular the line of identity

SD2: Poincaré plot standard deviation along the line of identity

SDNN: Standard deviation of NN intervals

SmO_2: Muscle oxygen saturation

T2DM, type 2 diabetes mellitus

TMA: Trimethylamine

TMAO: Trimethylamine N-oxide

TNF-α: tumour necrosis factor-alpha

URTI: Upper respiratory tract infections

VO_2: Volume of Oxygen

WFPB: Wholefood, plant-based

WHO: World Health Organization

WMD: weighted mean differences

Publications in support of this thesis

Peer reviewed publications

Craddock JC, Neale EP, Probst YC, Peoples GE (2020). A cross-sectional comparison of the whole blood fatty acid profile and omega-3 index of male vegan and omnivorous endurance athletes. *Journal of the American College of Nutrition* 25:1-9. doi: 10.1080/07315724.2021.1886196

Craddock JC, Neale EP, Peoples GE, Probst YC (2020). Plant-based eating patterns to improve endurance performance: A focus on inflammation, oxidative stress and immune responses. *Nutrition Bulletin*. 45(2), 123-32. https://doi.org/10.1111/nbu.12427

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Peer-reviewed publications under review supporting this thesis

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Other publications during doctoral candidature

Peoples GE, Parker S, Anthony R, Groeneveld T, **Craddock JC** (2021). Rock climbers' self-reported dietary practices and supplement use in the context of supporting climbing performance. *The Journal of Sport and Exercise Science.* 5(2):130-38. doi.org/10.36905/jses.2021.02.06

Craddock JC, Genoni A, Strutt EF, Goldman DM (2021). Limitations with the Digestible Indispensable Amino Acid Score (DIAAS) with Special Attention to Plant-Based Diets: a Review. *Current Nutrition Reports.* Online ahead of print DOI: 10.1007/s13668-020-00348-8

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1 Introduction

A substantial portion of this chapter has been published.

Craddock JC, Neale EP, Peoples GE, Probst YC, (2020). Plant-based eating patterns to improve endurance performance: A focus on inflammation, oxidative stress and immune responses. Nutrition Bulletin 45:123– 132. DOI:https://doi.org/10.1111/nbu.12427

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1.1 Vegetarian-based dietary patterns – An overview

Nutritional epidemiology has seen a shift away from single nutrient analyses to a complementary approach in the form of whole foods and/or dietary patterns [1]. Whilst single nutrient approaches can assist in providing biochemical and mechanistic assessment, this approach overlooks the role that dietary patterns may have in promoting health [2]. Evaluating dietary patterns offers a holistic and clinically relevant approach to assessing diet-disease relationships as nutrients are seldom eaten in isolation. Further, when foods are consumed together, as occurs in practice in the real world, there are synergistic effects between the multiple components which can have a concerted effect and are reflective of eating behaviours [3]. An array of dietary patterns exist, many of which have been shown to be associated with disease prevention such as the Mediterranean diet [4] and the Dietary Approach to Stop Hypertension [5]. Another dietary pattern which has been increasing in popularity is the vegetarian dietary pattern which will be examined extensively throughout this thesis.

Approaches to vegetarian-based dietary patterns range from the complete exclusion of animal products through to their inclusion at varying levels. For example, eggs or dairy may be consumed within vegetarian dietary patterns [6] referred to as ovo- and lacto-, respectively. Individuals adhering to vegan eating patterns typically exclude all products of animal origin, and avoid foods such as animal-derived milks, eggs and honey. Wholefood, plant-based (WFPB) dietary patterns also exist and appear to be particularly effective in protecting against chronic disease [7-15]. Variations of the WFPB diet exist, but they generally include unrestricted consumption of whole, plant-based foods including vegetables, fruit, grains and legumes, and the exclusion of; animal-based products, fatty and processed foods such as added oils, fried products and sugary packaged items. This thesis will use the term

'vegetarian-based' to collectively refer to, and encompass all types of vegetarian-based eating patterns where the majority of energy is consumed from plant food sources, while terms describing specific plant-based subclasses (for example, LOV, WFPB, ovo-vegetarian) will be used when appropriate. Table 1.1 provides an overview of the dietary patterns.

Table 1.1. Vegetarian-based dietary pattern classification

Type of dietary pattern	Definition	Red meat	Poultry	Fish	Dairy	Eggs	Processed foods
	Omnivorous						
Omnivorous/Mixed	Consumes red meat, poultry, fish, dairy and eggs.	✓	✓	✓	✓	✓	✓
	Vegetarian-based						
Pesco-lacto-ovo vegetarian	Consumes fish, dairy and eggs, but not red meat or poultry.	✗	✗	✓	✓	✓	✓
Pesco-vegetarian	Consumes fish but not red meat, poultry eggs or dairy.	✗	✗	✓	✗	✗	✓
Lacto-ovo vegetarian	Consumes dairy and eggs but not red meat, poultry or fish.	✗	✗	✗	✓	✓	✓
Lacto-vegetarian	Consumes dairy not red meat, poultry, fish, dairy or eggs.	✗	✗	✗	✓	✗	✓
Ovo-vegetarian	Consumes eggs but not read meat, poultry, fish, dairy or eggs.	✗	✗	✗	✗	✓	✓
Vegan	Consumes no red meat, poultry, fish, dairy or eggs.	✗	✗	✗	✗	✗	✓
Wholefood plant-based	Eliminates or substantially restricts red meat, poultry, fish, dairy, eggs and processed foods.	✗	✗	✗	✗	✗	✗

Precise historical data for vegetarianism and its origins are somewhat vague, although it is clear that most major human civilisations consumed a predominantly vegetarian-based diet [16]. Motivations for adhering to vegetarian-based eating patterns are vast with the primary motives including animal rights/welfare advocacy, environmental issues and social concerns, [16]. Today, despite the number of individuals following vegetarian dietary patterns reported to be on the rise, people consuming the diet remains a minority in most countries, except in India where approximately one third of the country's population follows a vegetarian diet [16]. In Australia, the prevalence of individuals following vegetarian-based dietary patterns is largely unknown. A survey has estimated that the adult population who identify as vegetarian or almost vegetarian in Australia was just under 2.5 million in 2018 increasing by ~11.2%

from 2.2 million in 2014. However, the data collected from this survey was self-reported and relied on individuals interpreting their own dietary intake patterns [17]. Likewise, there is a paucity of data exploring the nutrient intakes in those following vegetarian-based dietary patterns in Australia. Studies which do explore the nutrient intakes of vegetarian-based dietary patterns in Australia are generally dated, use small sample sizes and focus on a small selection of nutrients [18, 19].

Vegetarian-based eating patterns comply well with most dietary recommendations [20-22] and include more fruits, vegetables, whole grains, nuts, seeds and legumes than diets centered around meat and animal products [23-25]. Protection against many chronic diseases have been observed in individuals following vegetarian-based eating patterns including coronary heart disease, hypertension, diabetes mellitus, obesity and some cancers [12, 15, 26-32]. Individuals following vegetarian-based dietary patterns tend to have a favourable overall diet quality [33] and differing nutrient compositions [24, 34, 35] when compared to non-vegetarian based dietary patterns. Nutrients such as folic acid, vitamins C and E, fibre, magnesium, polyunsaturated fatty acids and many phytochemicals have been observed to be higher in populations following vegetarian-based dietary patterns [36-39]. These nutritional components have been shown to improve blood pressure [40], insulin sensitivity [41, 42], and can assist in the management of long-term weight gain [32]. Although, some nutrients such as calcium, vitamin B12, long chain omega-3 fatty acids (LC n-3 PUFA), zinc, and vitamin D may be harder to obtain when following a vegetarian-based dietary pattern which could lead to nutritional deficiencies and detrimental outcomes if the diets are poorly planned [43]. Of course, Accredited Practicing Dietitians can play important roles in ensuring nutrient adequacy for those following vegetarian-based diets which are suitable for all stages of the lifecycle when well-structured [44].

This chapter provides an overview of the relationship between vegetarian-based dietary patterns and their potential to influence markers of inflammation and immune function as well as how differences in nutrient intake between vegetarian and non-vegetarian diets may modulate exercise physiology. The literature is also reviewed outlining the current knowledge pertaining to vegetarian-based dietary patterns, exercise physiology and endurance exercise outcomes. Ultimately, this chapter describes the current gaps which will provide the focal point for the subsequent chapters within this thesis.

1.2 Endurance exercise and vegetarian-based dietary patterns

1.2.1 Endurance exercise

Several forms of exercise exist including strength, flexibility, balance and endurance. Among these exercise forms, aerobic training or endurance activity appear to provide the most favourable metabolic and cardiovascular benefits [45]. Endurance exercise is characterised by repetitive isotonic contractions of large muscle groups [45] such as swimming, running and cycling which has been on the rise in recent decades [46]. There are a range of health benefits associated with increasing one's aerobic capacity via endurance activity. For instance, a one metabolic equivalent (one metabolic equivalent (MET) equals the amount of oxygen consumed while sitting at rest and is equal to 3.5 mL O_2 /kg BW/ min^{-1}) increase in exercise-test performance induces a 12% improvement in overall mortality [47]. Myers et al. stated that maximal aerobic capacity is "a more powerful predictor of mortality among men than other established risk factors for cardiovascular disease (pg 793)" [47]. Furthermore, low aerobic capacity is an independent risk factor for type two diabetes mellitus and other chronic diseases [48].

1.2.1.1 *Physiological changes during endurance exercise*

Physiologically, endurance exercise requires a paring of both the respiratory and cardiovascular system to supply the contracting muscles with energy to perform mechanical work. The cardiovascular system provides oxygen rich blood to the skeletal muscles whilst simultaneously removing waste products such as lactic acid and carbon dioxide which requires an increase in cardiac output [45]. Whilst the cardiovascular system can be trained, the respiratory system is unable to improve its performance beyond its natural limits [49] although may increase ventilation rates 20 times whilst exercising compared to at rest [45, 49]. Other physiological changes occur as a result of endurance exercise. For instance, mitochondrial density can be increased, oxidative enzymes utlised in energy production can be augmented whilst muscle fibre type can be shifted with improved capillarisation observed, all of which influences muscle metabolism and work economy [45].

1.2.2 Diet and endurance performance

The role that nutrition can play in endurance exercise is well documented, although, in many areas scientific consensus is not always apparent [50]. Below some of the more accepted and utilised nutrition approaches with guidelines briefly summarised which have an ongoing presence throughout this thesis. Whilst general guidelines exist concerning the optimisation of nutritional intakes for the endurance athlete, an individualised nutritional approach for each individual athlete and related sporting modality are underscored [51]. While this thesis focuses on dietary patterns as a whole, previous research has focused predominantly on the effects of consumption of specific macronutrients, particularly carbohydrate and protein and warrants consideration.

1.2.2.1 *Carbohydrates*

Carbohydrates for endurance performance are one of the most researched nutritional components for endurance performance and have long been recommended for endurance athletes [52]. The role of carbohydrates is vast with its importance emphasised to improve recovery, reduce muscle damage and fuel activity [50]. Specifically, without provision of carbohydrates for the endurance athlete, prolonged submaximal and intermittent high intensity activity is hindered [53].

Suggested carbohydrate intake varies depending on many variables, including duration of activity, type of activity and intensity, timing of training or event, however, the following provides a generalised overview. It is suggested for those engaging in ~ 1 hour of moderate activity individuals should consume 5-7g/kg/day, those engaging in 1-3 hours day should consume 6-10 g/kg/day and those 4 hours or more per day 8-12 g/kg/day [50]. Further, the timing of carbohydrate consumption is important, especially for competition and enhancing performance with 30-60 grams carbohydrate recommended per hour for exercise <2.5 and, 60-70 grams carbohydrate per hour for exercise exceeding 2.5 hours. Athletes able to tolerate up to 90 grams of mixed carbohydrate (glucose and fructose) per hour during endurance exercise may increase oxidization rates which is associated with improved performance [50].

1.2.2.2 *Protein*

Protein intake traditionally attracts less attention for endurance athletes than carbohydrates, although adequate intake (and timing) is equally as important. Athletes require higher intakes of protein than that is recommended for the general population (0.84g/kg/day men, 0.75g/kg/day women for adults aged 19-70 years old) to facilitate training adaptions and enhanced performance [54]. Adequate protein intake with appropriate timing is essential for

the endurance athlete as it can assist in reducing muscle protein breakdown, delaying central fatigue, reducing upper respiratory tract infections and contribute to a net positive nitrogen balance [55]. Similar to carbohydrate recommendations, protein intake guidelines are also dependent on the individual's training schedule and background and, therefore, an individualised approach should be implemented for each athlete. In general, endurance athletes should consume between 1.2 – 2 grams of protein per kilogram per day. Additionally, 0.3g/kg of protein should be consumed within the two hours after training to promote muscle protein synthesis [50].

While there has traditionally been a strong focus on the consumption of macronutrients for endurance performance, recently, athletes have adopted vegetarian-based dietary patterns, perhaps believing that the diet may enhance endurance performance [56-58]. One study reported 34% of their ultra-endurance runner sample identified as either vegetarian or vegan [59]. Amongst ultra-distance runners, vegetarian-based diets have gained popularity perhaps due to high profile, professionals athletes following and advocating for these dietary patterns [60, 61]. However, there is a scarcity of evidence available exploring motives of endurance athletes choosing to follow a vegetarian-based diet. Further, few studies have explicitly examined the relationship between vegetarian-based dietary patterns and endurance performance [62]. Despite the scarcity of empirical literature evaluating vegetarian-based nutrition and its influence on endurance performance, there is theoretical evidence exploring this notion.

1.3 Vegetarian-based diets, inflammation and immune function

1.3.1 Inflammation and vegetarian-based dietary patterns

Inflammation is the body's initial response to a wide range of harmful stimuli which may be

caused by tissue damage, pathogenic microbes, or other irritants. It is a complex biological response used for protection and is associated with intracellular signaling molecules which can influence both immune and inflammation responses [63]. Research has demonstrated links between chronic low-grade inflammation and chronic disease risk, with inflammation hypothesised as an underlying pathophysiological mechanism. For instance, chronic elevation of the inflammatory markers C-reactive protein (CRP), Interleukin-6 (IL-6) and fibrinogen has been shown to predict risk of cardiovascular disease [64], type 2 diabetes mellitus [65] and some cancers [66]. CRP and fibrinogen are both acute phase proteins extensively used as markers of systemic inflammation [67], whilst IL-6 is a cytokine also associated with the acute phase response and inflammatory response [68]. Of course, acute inflammation can be healthful, protecting against harmful stimuli, responding to damaged cells and initiating healing processes, but may become deleterious if sustained [69].

Numerous studies have reviewed the influence of diet on inflammation with clear associations found between the two. Dietary patterns where substantial amounts of energy are obtained from plant sources compared to animal sources have been shown to attenuate chronic inflammation. For instance, Neale et al evaluated the evidence for the effects of healthy dietary patterns on markers of inflammation [70] finding that dietary patterns rich in whole grains, fruits and vegetables were associated with reduced CRP levels. Other studies have also demonstrated that dietary patterns rich in fruits, vegetables and whole grains are associated with low levels of circulating inflammation markers such as CRP, IL-6, and fibrinogen, while Western diets rich in meats have been positively associated with these biomarkers [39, 71, 72].

1.3.2 Immunity, diet, and exercise

The immune system utilises a range of defense mechanisms to combat infection, some of which include; maintaining epithelial barriers, production of mucous secreting cells to maintain physiological norms such as appropriate pH levels, initiating cell differentiation (immunoglobulins), upregulating proteins associated with the acute phase response, increasing cytokine production and the production of reactive oxygen species (ROS) [73, 74]. This process requires an adequate status of antioxidant enzymes, vitamins and peptides [75]. Without a sufficient exogenous supply of nutrients to perform the above tasks, the capability of the immune system will be jeopardised [74, 76].

'Non-nutritive' components of food and their beneficial impact on immune function have been acknowledged in the literature [77-79]. For instance, Mainardi et al [78] reported that the consumption of resveratrol and quercetin, two common polyphenols found in a range of fruits and vegetables, both positively influence cells implicated in immune function. Similarly, juiced fruit has been investigated given its high polyphenolic concentrations with improvements in lymphocyte responsiveness and natural killer cell functionality following its consumption [80]. Some flavonoids also exhibit anti-viral effects, and can modulate natural killer activity, macrophage responses, and are linked to reduced systemic inflammation [81, 82].

Further, a recent meta-analysis showed that flavonoid supplementation decreased upper respiratory tract infections (URTI) incidence by 33% compared to a control group [83]. This is of particular interest to the training athlete as they are often immunocompromised, which increases their risk for URTI [84]. Carotenoids have also been implicated in immune modulation, with Watzl et al [85] demonstrating that when participants refrained from consuming any fruits or vegetables for two weeks, T-lymphocyte function decreased, yet

when tomato juice was introduced (40 mg lycopene/day and 5mg b-carotene/day), T-lymphocyte function was restored.

Due to the high intake of some key nutrients and phytochemicals in groups following vegetarian-based diets, it is plausible that a difference in immune status may be observed between persons following vegetarian-based and non-vegetarian-based dietary patterns. Although, few studies have explored this notion, some studies have demonstrated lower leukocytes [86-90], lowered lymphocytes [91-93], lowered neutrophils [89] and lowered compliment factor 3 (CF3) [93] in those following vegetarian-based dietary patterns. Furthermore, natural killer (NK) cell activity of peripheral blood lymphocytes has been shown to be elevated in vegetarian-based populations compared to non-vegetarian-based populations [94] while others have found no difference in NK cell activity [93, 95]. The lowered leukocyte concentrations in those following vegetarian-based dietary patterns may be favourable, as elevated leukocyte biomarkers have been associated with increased risk of all-cause mortality, T2DM and metabolic syndrome [96-98]. If lymphocyte counts are reduced in populations following vegetarian-based diets, yet NK cell cytotoxic activity is increased, the overall effect on immune function may also be favourable. It is important to note that whilst these markers may be significantly reduced in vegetarian-based dietary patterns compared to non-vegetarian dietary patterns, they remain within normal reference ranges [99]. The clinical relevance of reduced CF3 in populations following vegetarian-based diets, if substantiated, is unknown; however, CF3 has been associated with a higher risk of developing T2DM and insulin resistance [100], weight gain [101] and cardiometabolic risk [102, 103]. Conversely, if concentrations of CF3 are too low, host protection against infection may be jeopardised. This was not observed in the study reporting lowered CF3 in vegetarian-based dietary patterns as functional immunocompetence did not differ between dietary groups measured by

mitogen stimulation and NK cell cytotoxic activity [93].

1.4 Vegetarian-based diets and exercise related physiology

1.4.1 Inflammation, vegetarian-based dietary patterns and potential exercise physiology modulation

Considering that plant-derived foods are associated with lowered baseline inflammatory markers, it is plausible that differences in cytokine production during endurance performance may exist between individuals following vegetarian-based dietary patterns high in these food components. Since elevated IL-6 levels have been theorised to be one of the most sensitive biochemical markers for skeletal muscle inflammation, fatigue and metabolic turnover [63], there may be differences in the inflammatory response during endurance performance between vegetarian-based and non-vegetarian-based groups, as baseline IL-6 is lowered in those following vegetarian-based diets [72]. Interestingly, a major stimulus for increased IL-6 production is reduced muscle glycogen stores [104, 105]. Intakes of carbohydrates for those eating vegetarian-based dietary patterns are typically higher than other dietary patterns and have been reported to increase muscle glycogen concentrations which may improve endurance performance [106, 107]. Increases in IL-6 during exercise has also been reported to increase lipolysis [108], oxidation rate [108] and hepatic glucose production [109]. To date however, no data is available comparing IL-6 concentrations in endurance athletes following vegetarian or omnivorous diets.

While anti-inflammatory outcomes have been observed in vegetarian-based dietary patterns, likely due to the effects of the array of phytochemicals and other compounds present in the diets. There have also been pro-inflammatory observations in groups consuming dietary patterns high in meat [110]. One molecule which has been linked to pro-inflammatory

pathways is Trimethylamine N-oxide (TMAO) [111-113]. Trimethylamine (TMA) is metabolised from choline, betaine, ergothioneine and carnitine via gut microbiota, and further oxidised to form TMAO [113]. Choline and carnitine are predominately found in animal products such as eggs and red meat [111, 114]; however, production of TMAO from these molecules in populations following vegetarian-based eating patterns is substantially reduced when compared to omnivorous eating patterns due to differences in the gut microbiome [111].

Dietary lipid consumption can modulate inflammatory pathways via intracellular signaling, synthesis of bioactive lipid mediators such as prostaglandins, and activation of transcription factors for gene expression [115, 116]. A recent review evaluated the evidence for the relationship between lipid intake and inflammatory gene expressions, concluding that saturated fatty acid consumption is positively correlated with many pro-inflammatory pathways including IL-1,IL-6 and TNFα [117]. Further, CRP concentrations have been independently correlated with increased dietary saturated fatty acid intakes [118]. Contrary to this, omega-3 fatty acids have also been shown to modulate some inflammatory and immunological pathways. Substantial evidence from observational and human intervention studies link the long-chain omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) to anti-inflammatory actions [119, 120] and improved cell-mediated immune function [116]. Several studies have reported improved recovery with reduced muscle soreness following DHA/EPA supplementation in untrained athletes, although, this was following eccentric exercise [121-123]. Interestingly, lower intakes of preformed DHA and EPA are consumed in vegetarian-based eating patterns with lower omega-3 indexes observed in these groups compared to omnivorous populations who consume fish and animal products [124]. The lowered omega-3 index observed in these

groups; including those following a WFPB diet may negatively influence exercise induced stress markers if the athlete fails to; consume adequate preformed long chain omega-3s in algal forms (DHA/EPA), consume insufficient alpha linoleic acid (ALA) which can be converted to DHA and EPA or balance consumption of omega–6 to omega–3 fatty acids (ideally ~3:1 respectively for optimal conversion; [125]).

This section has discussed several individual nutrient and physiological pathways which may influence various inflammatory processes providing a potential mechanism of vegetarian-based dietary patterns to modulate inflammation. It is important, however, to consider these isolated events with the summative context of a vegetarian-based diet. As vegetarian based diets are low in choline, betaine, carnitine and saturated fat while optimising unrefined carbohydrate and phytochemical intakes, small improvements in inflammatory pathways may be observed in individuals following these dietary patterns which could have flow on effects for endurance performance.

1.5 Immune markers, vegetarian-based dietary patterns and potential exercise physiology modulation

Limited literature exists exploring the differences between vegetarian-based and non-vegetarian-based dietary patterns and how the differences in immune function may influence endurance performance specifically. One such study which has explored this concept was that of Richter et al [95] who found no difference in maximal aerobic capacity between a LOV diet and meat-rich Western diet after a 6-week cross over study with trained athletes. This study did not find differences in blood mononuclear cells or NK cell activity. The authors noted that they collected blood from participants at rest and advised participants not to engage in any strenuous activity in the 24 hours prior to blood collection. This negates the

acute effects of the immune response to exercise. The trial concluded that the immune markers may be different between the two dietary patterns when observed during exercise.

It is plausible that due to the variations in nutrient concentrations between vegetarian-based and non-vegetarian-based dietary patterns, vegetarian based dietary patterns may favourably modulate immune function [89, 94, 126]. It is recognised that high intensity and endurance exercise can cause immunosuppression due to the prolonged and intense activity in which there is an 'open window' where pathogens have an increased likelihood of invading a host [127]. It is reasonable, therefore, to hypothesise that those athletes following vegetarian-based diets may have increased protection against pathogens following heavy training periods, due to their high consumption of antioxidant rich foods, especially carotenoids and polyphenols in the wider context of a vegetarian-based diet. This could practically translate into less illness with more time spent training for athletes following vegetarian-based diets, but further research is required exploring this notion.

1.6 The effect of vegetarian-based dietary patterns on other aspects of exercise physiology

Other hypothetical mechanistic pathways of vegetarian-based dietary patterns and the potential to modulate exercise physiology exist. These include: blunting oxidative stress, increased carbohydrate intake leading to improved glycogen storage, leaner body mass and improved tissue oxygenation although, these factors have scarcely been investigated in clinical studies though an overview is provided below.

1.6.1 Blunting aerobic stress

Aerobic exercise has been shown to elicit oxidative stress [128, 129]. One of the physiological underpinnings of increased oxidative stress during physical activity is due to the increased oxygen requirements by skeletal muscles. This can cause electrons to 'leak'

from the mitochondria resulting in an increased production of free radicals and lipid peroxidation with oxidative stress ensuing [129, 130]. Oxidative stress has been linked to cell damage, a range of pathologies including neurodegenerative diseases and atherosclerosis, muscle fatigue and overtraining, all of which can impact upon endurance performance [131, 132]. At rest, the cellular antioxidant system is able to remove these potentially harmful free radical molecules; however, when there is an imbalance between the production of free radicals and antioxidant defenses such as when an individual is exercising, oxidative stress can result [133]. While intense and excessive endurance training can elevate levels of free radicals acutely, adaptations in the body's antioxidant defense system can result following sustained aerobic and anaerobic exercise training over time [134, 135].

Dietary patterns and foods rich in antioxidants such as vitamin C, vitamin E, polyphenols and beta-carotene have been shown to improve a person's antioxidant status [133, 136]. Polyphenols are the most abundant antioxidants consumed in the diet [137] with particularly high intakes observed from vegetarian-based dietary patterns [138]. The high dietary intake of polyphenols in vegetarian-based dietary patterns has been reported to translate to an enhanced antioxidant status for those following a vegetarian-based dietary pattern [136, 139, 140].

Much of the research surrounding polyphenols consumption and enhanced endurance performance via improved antioxidant status has focused on polyphenol rich extracts rather than whole dietary patterns or whole plant foods. A recent review focusing on polyphenol-rich plant extracts and their ability to modulate exercise induced stress reported a small but significant increase in antioxidant capacity following consumption of these extracts; however, this increase in antioxidant capacity inconsistently mitigated exercise-induced

oxidative stress, inflammation, and immune dysfunction [79]. Another study examining antioxidant intake on exercise capacity was that of Fogarty et al., who examined the influence of watercress; a cruciferous vegetable high in antioxidants including beta-carotene and α-tocopherol. Fogarty et al demonstrated that watercress could attenuate DNA damage and lipid peroxidation whilst reducing hydrogen peroxide accumulation following exhaustive exercise [141].

Although the aforementioned studies examined plant-based extracts or individual foods on antioxidant status, a review exploring the effects of vegetarian-based dietary patterns compared to non-vegetarian-based dietary patterns on antioxidant status and oxidative stress in response to anerobic and aerobic exercise, reported that the evidence base was limited and inconsistent [142]. While the authors highlighted that much of the existing research examines the effects of supplements on antioxidant status rather than whole dietary patterns, they concluded that persons following vegetarian-based dietary patterns may have a superior antioxidant status, and be better equipped to negate exercise induced oxidative stress [142]. This position was echoed by Yavari et el who reported that supplementing with specific antioxidant products may result in inconsistent effects on oxidative stress and recommended that consumption of whole foods rich in natural phytochemicals are best for optimal antioxidant status [143].

As vegetarian-based dietary patterns typically provide regular antioxidant dense polyphenols which can modulate antioxidant status [139, 140], it follows that these dietary patterns may reduce exercise induced oxidative stress, although more research is required.

1.6.2 Glycogen storage

Muscle and liver glycogen storage play a crucial role for the endurance athlete in optimising performance as glycogen is used as fuel for aerobic activity. Carbohydrate (CHO) availability prior to, during and post competition or training session can increase both liver and muscle glycogen stores during recovery, while provide energy for the working athlete [52]. Research has consistently shown that the consumption of CHO to maintain or increase CHO concentrations have been shown to prolong endurance exercise capacity by postponing the onset of fatigue [53, 144]. Underconsumption of carbohydrates in endurance athletes is not uncommon [145]. For example, in a study of 116 non-elite endurance athletes only 45.7% of athletes achieved the suggested CHO requirements (≥6 g/kg body weight per day) [146]. Since vegetarian-based dietary patterns are associated with greater CHO intake in general population groups [36, 38] and within athlete population groups [147, 148] compared to those following omnivorous dietary patterns, it has been suggested that those adhering to a vegetarian-based dietary pattern may augment their glycogen status affording a performance advantage by delaying the time to fatigue [107, 149].

1.6.3 Lean body mass

Vegan dietary patterns are typically less energy dense than omnivorous dietary patterns resulting in lower BMI and favourable body composition for the endurance athlete [32, 107]. Lower total body fat is associated with improved submaximal and maximal aerobic capacity [150] and importantly in athletes matched for maximal aerobic capacity, those who are of lower body weight will outperform those of higher weights [107] which highlights the importance of optimising weight control within endurance athletes. The reduced body fat composition observed in groups following vegetarian-based eating patterns has been attributed to the high fibre, low fat composition typically observed in the diet which consequently reduces total energy density [28]. Notably, research has indicated that low fat vegetarian-based

diets may influence mitochondrial activity by increasing the number and activity of the organelles thereby increasing postprandial energy expenditure [7] contributing to lean body body mass.

1.6.4 Tissue oxygenation

Consumption of a low fat vegan dietary pattern may reduce blood viscosity via reduced plasma lipid concentrations [34], improve atrial compliance and improve endothelial function [151, 152]. As endurance activity relies on good circulation to deliver oxygen to contracting muscles, and eliminate metabolic waste products, it has been hypothesized that adherence to a vegetarian-based dietary pattern may improve vascular flow and tissue oxygenation thereby promoting an ergogenic endurance effect [107] due to their naturally low fat composition.

1.7 Effect of vegetarian-based dietary patterns on endurance performance

Empirical evidence testing vegetarian-based diets and endurance performance is scarce, and as a result, much of this chapter has discussed the physiological underpinnings as to why a vegetarian-based diet may improve endurance performance. However, a small number of studies directly examining the impact of consuming vegetarian-based diets on endurance performance do exist. Veleba et al reported significant increases in maximal oxygen consumption (12%) and workload (21%) in participants with type 2 diabetes adhering to calorie restricted WFPB diet for 12 weeks. In comparison, no increases were observed in participants consuming a calorie restricted conventional type 2 diabetic diet [153]. The authors concluded that consuming a WFPB diet may produce superior improvements in "physical fitness" compared to conventional diets when following an aerobic exercise program. It was proposed that the WFPB group was better able to utilise carbohydrates whilst exhibiting increased insulin sensitivity which may partly explain the improvements in performance compared to the conventional type 2 diabetic diet. In a recent study examining

the effects of a vegan diet on physical activity a vegan dietary pattern was shown to invoke significantly higher submaximal endurance time to exhaustion (12.2 ± 5.7 vs. 8.8 ± 3.0 min; p = 0.007) at 70% MaxVO_2 compared with consumption of an omnivorous diet in active individuals [149]. However, in another trial comparing LOV dietary patterns to meat-rich Western diets in trained athletes, no differences in maximal aerobic capacity were observed after a 6-week cross over study [95, 154]. Other studies have investigated vegetarian-based dietary patterns and endurance performance, but are case studies [155, 156], or cross sectional with small sample sizes [147, 157, 158]. Deriving conclusions pertaining to the ability of a vegetarian-based diet to modulate endurance performance from these studies would be disingenuous as natural differences in physiology between groups could influence the results irrespective of diet.

1.8 Summary of evidence and gaps in the literature

Consumption of vegetarian-based dietary patterns is associated with improvements and protection against many chronic diseases and has largely been attributed to their food components such as fruits, vegetables, whole grains, nuts, seeds and legumes and absence of animal foods. However, the synergistic effects of these foods consumed within a vegetarian-based dietary context on immune and inflammatory status has not been collectively assessed. This thesis will therefore explore the relationship between vegetarian-based diets and markers of inflammation and the immune system.

Despite the theoretically proposed underpinnings of a vegetarian-based dietary patterns ability to influence exercise physiology including inflammation and immune function, limited research has been conducted in this domain. Even less research exists examining the endurance athlete's nutrient intake and diet quality in those following a vegetarian-based

dietary pattern. Differences in dietary composition between athletes following omnivorous and vegetarian-based dietary patterns may translate into various exercise related physiological outcomes including inflammatory and immunological.

1.9 Significance of research

This thesis will be the first, to the candidate's knowledge, to explore the dietary patterns, motives, diet quality and nutrient status within athletes following a vegetarian-dietary pattern. Understanding the athlete motives are important for several reasons and have implications for individuals, health professionals and researchers. Gaining insight into the motives and behaviours may assist in informing ways to improve individual dietary patterns and overall health, be used to reveal differences in various dietary outcomes among individuals and groups and also may be used to inform public health nutrition messages for athletes following this type of diet.

Examining the relationship between vegetarian-based dietary patterns and markers of inflammatory and immune function will have relevance for the health of individuals following this dietary pattern. Chronic low-grade inflammation is associated with increased risk of many chronic conditions [159] and, therefore, this research may offer lifestyle strategies to help ameliorate inflammation and improve immune function. Identifying differences in these markers between dietary patterns may also illuminate potential physiologically relevant exercise markers enabling individuals to make informed decisions regarding their nutritional intake and improve exercise performance outcomes. Further, this research will provide a foundation contrasting and examining if nutritional differences as part of a vegetarian-dietary pattern can modulate exercise physiology. This is particularly

important as more athletes begin to adopt a diet of this nature [160].

This thesis will also have relevance for health professionals and clinicians by providing a more holistic understanding of the evidence for athletes following vegetarian-based diets, and how they might improve person centered care in this population.

1.10 Thesis aims and hypothesis

The central aim of this thesis is to explore potential differences in dietary intake between individuals following vegetarian-based or omnivorous dietary patterns and to determine if dietary intakes translate into dissimilarities in inflammatory, immune and exercise related physiological biomarkers. This central hypothesis is that due to differences in nutrient composition intake between vegetarian and non-vegetarian-based dietary patterns, disparities will exist in various biomarkers between groups, which will influence exercise physiology, particularly during endurance exercise.

The individual study aims were to:

- explore dietary behaviours, motivators and nutrition supplementation in self-reported athletes ranging from recreational to elite following self-reported vegetarian-based eating patterns.
- assess the nutrient status and overall diet quality of athletes self-reporting to follow a vegetarian-based dietary pattern.
- examine if vegetarian-based dietary patterns in humans are able to modulate inflammation and/or immune biomarkers compared to those following non-vegetarian dietary patterns.

- explore dietary fatty acid intakes and the relationship with whole and red blood cell fatty acid profiles in athletic populations consuming a vegan or non-vegan eating patterns.
- assess the habitual dietary quality and nutrient status of trained endurance athletes following either a vegan or non-vegan dietary patterns.
- investigate if differences exist between physiological outcomes i.e., cardiometabolic parameters and inflammatory biomarkers in a group of aerobically matched individuals consuming vegan or omnivorous dietary patterns during controlled exercise conditions.

1.11 Thesis overview and structure

This chapter has provided an overview and background information on vegetarian-based dietary patterns and how these patterns may modulate various nutrient compositions which could influence physiological systems resulting in exercise related physiological outcomes.

Chapter 2 will describe the methodological underpinnings for this thesis which includes a discussion of the evidence underpinning the methods used for each study. Strengths and weaknesses of the methods used are considered with justifications for methods that best addressed the central aim of the thesis. The thesis and study overview can be seen in **Figure** 1.1**.**

Chapter 3 (Study 1a) outlines the results of an exploratory survey investigating dietary behaviour, motivations and supplementation use in self-reported athletes following vegetarian-based dietary patterns.

Chapter 4 (Study 1b) presents the nutrient status and overall diet quality of athletes self-reporting to follow a vegetarian-based dietary pattern providing a rationale for exploring the biological/physiological markers used in studies 2 and 4.

Chapter 5 (Study 2) outlines a systematic review and meta-analysis exploring vegetarian-based dietary patterns and their relationship with inflammatory and immune biomarkers. This study was relevant to determine if a hypothesised difference in nutrient intakes between those following vegetarian-based dietary patterns and omnivorous dietary pattern translated which was observed in Study 1b translated into differences in biological markers which could be implicated in exercise physiology for Study 4.

Chapter 6 (Study 3) presents the dietary differences in trained endurance athletes following either vegan or omnivorous dietary patterns. Evaluating nutrient and quality differences between dietary groups is paramount to understanding the potential differences in exercise related physiology should they exist for Study 4.

Chapter 7 (Study 4) explores the exercise related physiology between matched endurance athletes following either vegan or omnivorous dietary patterns matched for age, training volume and maximal aerobic capacity.

Chapter 8 provides a summary of the main findings from the previous chapters in relation to the central aim and hypothesis of the thesis. Recommendations for future research in addition to the thesis strengths and weaknesses are also discussed.

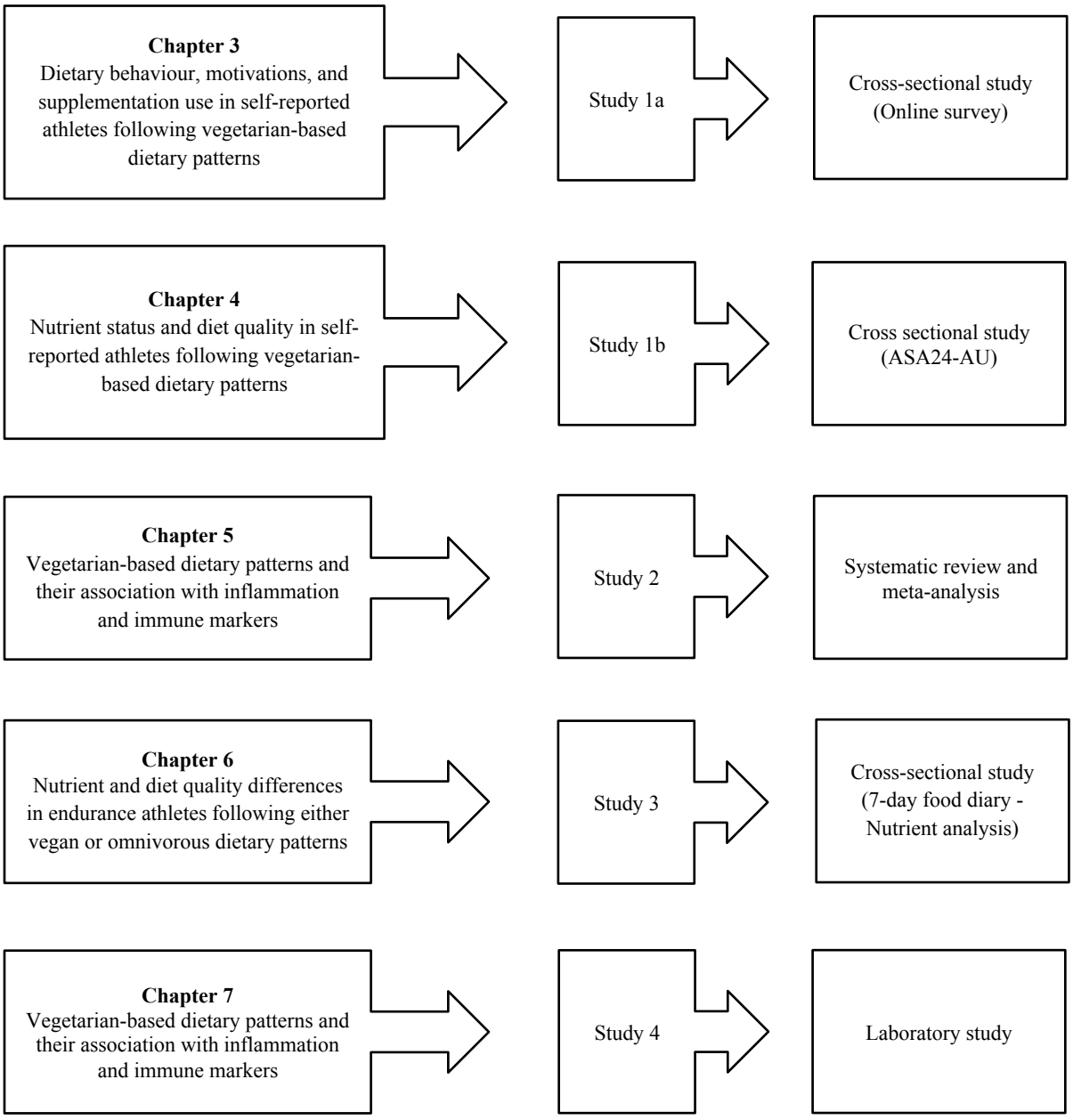


Figure 1.1. Overview of thesis and study designs

2 Methodology

2.1 Overview of methodological framework

For athletes following vegetarian-based dietary patterns, limited research exists examining their dietary behaviours, motives and nutrient status which may have a direct relationship with exercise related physiology as well as other health related outcomes. As noted in chapter 1, vegetarian-based dietary patterns have been associated with protection against many chronic diseases, yet the influence of the dietary pattern to influence systemic inflammation and immune function is yet to be synthesised. The nutrient interactions and diet quality for those following vegetarian-based diets may be responsible for modulating inflammatory and immune markers. To address the central hypothesis of this thesis, it was paramount to consider the various methodological approaches available. This chapter will, therefore, consider the approaches required to address the research aims proposed in this thesis providing a rationale as well as a critique of the methods used. An overview of the methods appraised include: online surveys, systematic reviews and meta-analyses, dietary assessment methodology, diet quality assessment tools, controlled laboratory based studies as depicted in Figure 1.1.

2.2 Online survey methodology

Individuals differ in their motives for following a vegetarian-based diet with the two most common motives being ethics (i.e. animal rights) and personal health [161]. Understanding the motives for abstaining from consuming meat and animal products is important to consider from a research perspective as individuals choosing to follow a vegetarian-based dietary pattern are likely to view their food choices and behaviours as a central component of their self-identity often with an array of motivations embedded in their self-understanding [161]. From a nutrition perspective, understanding one's motives for following a particular vegetarian-based dietary pattern is also crucial to consider for researchers as the literature suggests that one's motives may dictate one's dietary quality and in turn one's behaviours [162]. As such, considering motivations in the context of dietary patterns is of particular importance. Observational studies provide a viable methodology to explore motivations and dietary behaviours.

Several types of observational study designs exist including cross-sectional, cohort and case-control studies [163]. Case control studies are generally retrospective in nature whereby participants with and without the outcome of interest are matched. The researcher is then able to retrospectively compare variables to determine if any associations exist between the groups and the outcome of interest [163]. On the other hand, cohort studies typically follow a group or groups of participants over a period of time who usually share common characteristics such as a particular demographic. During the cohort study some of the participants will become exposed to various risk factors or characteristics which can then be compared to those who were not and subsequently used to compare to the outcome variable [164]. Cross-sectional studies differ to case-control and cohort studies as both the exposure and outcome are measured concurrently providing a 'snapshot' in time [165]. There are several strengths to

using a cross-sectional study design as they are generally simple, fast and easy to conduct while well suited to collect data in large populations [166]. On the other hand, they can be prone to selection and information bias and are unable to permit distinction between cause and effect [163, 167].

The most common forms of data collection in cross-sectional study designs are surveys or questionnaires [168]. Cross-sectional surveys permit data to be collected in a standardised format and can be self-administered or conducted by an interview [169]. Online surveys are increasingly being used in a self-administered survey form, perhaps due to their potential reach, cost-effectiveness and ease of automated data collection, especially when compared to paper-based self-administered or interviews based surveys [170]. Additionally, online surveys are flexible in nature and have been shown to produce honest responses in research areas with sensitive information [171] such as weight and eating patterns which were themes explored in this thesis. By contrast, there are some concerns with online surveys including ambiguity over the validity of data and sampling issues relating to the survey design and implementation [171].

When designing and implementing online surveys, it is, therefore, important to consider the associated limitations inherent to survey research. For instance, the type of questions included in the survey can influence the results of the survey. Open ended questions allow participants to respond in their own way, thereby increasing validity, however, are considerably harder to answer, take more effort and time to answer and are harder to analyse for the researcher [172]. Consideration is also required to ensure questions are constructed so that all respondents can understand and interpret the question equally and are able to respond accordingly [172]. Pilot testing of survey questions can assist in determining if the question

meanings are clear to help avoid misinterpretation.

Effective surveys rely on representation from the entire group being studied. The response rate is one metric which can provide information on the representativeness of the sample with high responses rates desirable. Generally, lower response rates for a survey increases the risk that the study respondents will differ from the non-respondents characteristics thereby limiting the reliability and generalisability [173]. It is, therefore, vital that surveys are designed to optimise potential response rates. Several methods can be embedded within survey design to assist in increasing response rates which include mode of survey delivery, survey length, personalisation, survey timing, follow up reminders and use of incentives [172, 174]. Several biases can also influence the accuracy of the survey outcomes. Non-response bias can occur when there are differences between respondents and non-respondent characteristics, yet this is not reported. Sensitive information bias is a respondent's willingness to disclose personal information about themselves and instead give socially desirable answers to skew survey results [174].

In the case of this thesis, an online self-administered survey was selected as this method enabled access a large group of participants who were following plant-based dietary patterns (Chapter 3). This was especially evident as athletes adhering to these dietary patterns in large numbers would have been difficult to recruit locally. Research has shown that online survey recruitment via social media sites such as Facebook has been successful for recruiting 'hard-to-reach' populations who may be interested in health-based research but are inaccessible through traditional methods [175]. Additionally, online surveys are ideal for attracting a large diversity of respondents in the sample by encouraging recipients who share the same outcomes of interest to the researcher to 'on-share' the survey [176]. In this thesis this 'on-

sharing' was encouraged to increase the sample size building upon Parkinson's and Bromfield's research [177].

A large component of the survey design in this thesis focused on increasing response rates as this has been described as one of the challenges for online surveys [178]. In this thesis, the option to skip questions was provided to respondents or choose non-responses, such as "not applicable" or "don't know" which has been shown to increase response rate [179]. The survey was developed with construct and face validity investigated by a convenience sample of seven experienced researchers where the researchers rated each survey item for relevance, accuracy, clarity and appropriateness on a 4-point Likert scale as part of the Content Validity Index (CVI) [180]. Additionally, each rater was asked to provide feedback regarding, ease of instructions, technical issues and time taken to complete the survey, as improving these aspects can reduce non-response rates [181]. The survey was developed using a mix of fixed and open-ended questions again to improve the likelihood of respondents completing the survey in its entirety [182]. Reminders were provided via email on three occasions with varied timing to increase the likelihood of responses and reduce non-response error [174].

2.3 Dietary assessment methods

To explore the nutrient status of self-reported athletes following vegetarian-based diets, dietary data was collected via a dietary collection assessment tool in this thesis. Appropriate dietary assessment tools to use depend on many variables including the research design, resources available, the research hypothesis and objectives [183]. With each dietary method there are trade-offs between time, cost, feasibility and accuracy required [183]. Whilst several dietary collection tools exist (diet history interviews, dietary recalls, food records and food frequency questionnaires), only the ones used within this thesis will be described with their

strengths, weaknesses and justifications provided.

2.3.1 24-hour diet recalls

Of the many dietary collection tools that exist, the 24-hour diet recall is one of the most frequently used [184]. The 24-hour dietary recall is a subjective, retrospective dietary collection method which has historically required face-to-face or telephone based interviews by trained professionals to quantify intakes of foods and beverages consumed within the previous 24-hour period [184]. The 24-hour dietary recall can provide good estimates of short-term total dietary, nutritional and supplement intake. Further, multiple 24-hour dietary recalls can be used across several time periods and non-consecutive days in order to provide usual intake in a population group. For example, the multiple source method is an approach used to statistically model usual intake by combining the probability and amount of consumption with covariates [185]. Additional relevant dietary information is also able to be collected such as meal timing and frequency, brands and locations of the foods consumed [186]. Several studies have also shown the improved accuracy for collecting dietary data using 24-hour recalls when compared to other dietary collection tools such as Food Frequency Questionnaires (FFQs) [187, 188]. On the other hand, there are several limitations. Most notably, 24-hour recalls are unsuitable for collecting distant past meals, and single 24-hour recalls may not capture irregular consumed foods thereby may under or overestimate some nutrients. Traditional 24-hour recalls may also place a large burden on the research team with considerable input required to collect the recalls and manually code the data which requires training and quality assurance components [186, 189].

Self-administered web-based versions of 24-hour dietary recalls have been created and used extensively with one of the key advantages of the tool being self-administered thereby

minimising cost and increasing flexibility to complete by the participants. The are several advantages for collecting intakes via a web-based 24-hour dietary recall including: the ability to administer the recall quickly and easily [190], the retrospective nature of the tool means participants intake is unlikely to be altered [184] and there is an increased response rate compared to other dietary tools [184].

The Automated Self-Administered 24-hour Recall (ASA24-AU) [191] is a self-administered web based 24-hour dietary recall tool and has been shown to have very similar results when compared to the interviewer-administered 24-hour dietary recalls [192, 193]. The ASA24-AU is underpinned by Australian Food, Supplement and Nutrient Database (AUSNUT) 2011-13 with foods reported in the ASA24-AU linked to AUSNUT food and measure codes for nutrient calculations. The ASA24-AU limits the expenses of administration and coding which are traits historically associated with interview based 24-hour dietary recalls. Importantly the validity of the ASA24-AU has been tested with acceptable face validity in terms of energy, nutrient, and food group estimates [194, 195]. There are limitations to this methodology which should be noted. Firstly, as with all 24-hour recalls, the ASA24-AU only collects information about an individual's intake across a 24 hour time period and, therefore, may not accurately reflect usual intake, likely missing foods that are consumed regularly, but infrequently [183]. Secondly as the tool is retrospective in nature, it places extensive emphasis on the participant's ability to recall their intake and may not be suitable for children or the elderly [184]. However, as the sample size was anticipated to be large (>100), predominately composed of middle-aged participants anticipated to be dispersed geographically, due to the accuracy of dietary data collected, and ease and cost to implement, the ASA24-AU was deemed to be well suited to collect the dietary intake in Study 1b (Chapter 4).

2.3.2 Food records

A food record involves participants recording all food and drinks consumed in a given period, typically 3-7 days and includes one weekend day [190]. Intake can be recorded as estimated portions or weighed/measured with the latter providing a more precise measurement [190]. Food records can reduce reliance on participant's memory to recall their intake and instead record their intake in real time. This has contributed to food records having a high validity [183, 196]. Food records are considered a suitable dietary assessment methods in both clinical and epidemiological settings [183, 197] whilst also often utilised as a reference for validating other dietary assessment methods [198].

On the other hand, food records are reliant on participant motivation and may become inaccurate if motivation is low and the burden is placed on them to complete the food record [183]. Food records may also take considerable time and effort to convey the food data to a nutrient analysis platform by a trained researcher [199]. Notably, as the duration of recording extends, the dietary tool becomes less valid as participant fatigue can develop. As the participants in this thesis were self-selected and interested in exercise and nutrition it was likely that the participants would be highly motivated to complete a food record and was deemed appropriate for the experimental trial. A 7-day food record was utilised in the laboratory Study (chapter 5) examining dietary intake and nutrient status.

2.4 Diet quality indexes (DQIs)

Diet quality indexes are research 'tools' typically used to measure the diet quality and are often based on nutrients, food groups or dietary guidelines and are underpinned by current nutritional knowledge [200]. In effect, DQIs represent standards of general 'healthfulness' of a pattern of intake [201]. DQI's vary widely and can be general in nature, for example the

Healthy Eating Index whereby the tool aims to track dietary guidelines, or can be specifically used to measure adherence to a particular dietary pattern such as the Mediterranean diet (MedDiet) food checklist [202].

Selecting the right DQI tool is paramount to predicting health related outcomes. Tools have inherent advantages, disadvantages and related considerations [203], for example, selecting a DQI that assesses nutrients and food or food-group based dietary indices may be advantageous as it is more likely to capture the complexities of food intake patterns, in addition to nutrient and non-nutrient components of a dietary pattern [204]. There is also sizeable difference in the components embedded within various DQIs, as well as deviation between country specific dietary guidelines. Some researchers have consequently questioned the utility of DQI's as health outcome predictors [205, 206]. Further, DQIs are typically easy to compute providing a good overall reflection of diet quality, their scores are quantitative and do not provide descriptive information of food intake patterns [201] though care should be taken when translating outcomes. Despite the considerations DQIs are seen to be relevant, effective and valid tools to link dietary pattern consumption with health outcomes available and, providing the limitations are acknowledged when interpreting results, remain a useful instrument [207, 208].

As one of the hypotheses of this thesis was that those who are following vegetarian-based dietary patterns may have favourable inflammatory markers, a diet quality tool that correlated to inflammation was required. The Alternate healthy eating index (AHEI-2010) is a validated dietary quality index tool which has a strong inverse correlation with inflammatory markers [209] and chronic disease risk [210] and was, therefore, selected for this thesis. Furthermore, the AHEI-2010 does not discriminate against either population group (vegetarian or non-vegetarian based) under investigation in this study. This differs from other published tools,

for example, the Diet Quality Index-International (DQI-I) which awards points within 'variety' scoring criteria for including all of the following in the protein category: meat, poultry, fish, dairy, beans, eggs. Individuals following a vegan based diet would only include beans from this list, though may source a variety of beans, legumes and tofu which the DQI-I does not incorporate. Additionally, the AHEI is developed to explore intake of both foods and nutrients enabling examination of dietary patterns and nutrient quality.

The Dietary Phytochemical index (DPI) was also selected to assess the dietary patterns in both Study 1b and 3 as differences in inflammatory markers are thought to be observed between the dietary groups. A possible mechanism for this difference may be explained by the high concentration of phytochemical intakes typically characteristic of vegetarian dietary patterns [211]. The DPI recognises the role that phytochemicals can play in modulating physiological processes including their antioxidant capacity, and their ability to reduce inflammation [212]. The DPI provides a percentage of dietary calories supplied by foods typically high in phytochemicals including: whole grains, vegetables, legumes, nuts, fruits and seeds.

Whilst there are thousands of dietary phytochemicals, the DPI includes foods generally considered to be abundant in any type of phytochemical in its calculation [213]. Of course, this approach is only able to provide an approximation of total phytochemicals consumed, but its application has two important strengths. Firstly, it acknowledges the synergistic effects whole foods abundant in phytochemicals may have, enabling epidemiologists to roughly correlate phytochemical with various health outcomes. This is important in the context of this thesis as it is examining a vegetarian-dietary pattern and is interested in the additive effects of the diet. Secondly, it can provide a snapshot of an individual's or cohort's diet quality identifying the need for more phytochemical rich foods to be consumed. Of course, as the

DPI is only an indication of phytochemical intake it lacks specificity and fails to consider the superior health benefits of some phytochemicals over others. Nonetheless the DPI is a useful tool for providing an approximation of consumption of phytochemical dense foods and their synergistic effects, important for this thesis.

2.4.1 Physical activity assessment

Assessing physical activity within the online survey was an important feature as increased physical activity generally requires increased energy consumption which has direct links to dietary behaviors and nutrient intake. To assess physical activity levels the International Physical Activity Questionnaire-Short Form (IPAQ-SF) was implemented [214]. The IPAQ-SF is comprised of seven components which focus on time spent during walking, or engaging in moderate or vigorous physical activities as well as the frequency of these during a 7-day period prior to the questionnaires administration. The IPAQ has been designed for use in adult populations aged between 18-69 years and can provide an estimation of physical activity patterns across different countries and socio-cultural contexts [214], which was relevant for this thesis as the survey was internationally distributed. Two versions of the IPAQ exist, both of which have valid and reliable results, although the IPAQ committee has suggested the short IPAQ-SF be used to compare national and international prevalence due to its shorter application time and ability to capture total physical activity [215]. The IPAQ-SF was, therefore, used in this thesis for Study 1a.

2.5 Systematic reviews and meta-analysis methodology

Systematic reviews (SR) provide a rigorous assessment of the available literature relating to a research question of interest. A standardised methodology exists for each component of a SR including searching, screening, reviewing, critiquing, interpreting, synthesising, and reporting

the findings to ensure that the most accurate assessment of the literature is presented [216].

SR's apply criteria which assist in the synthesis of relevant literature, often used to inform clinical decisions and promote evidence-based practice [217]. To help facilitate evidence-based practice, it is recommended that studies are evaluated by their study design which dictates a level of evidence [218]; with randomised controlled trials ranked the second highest (level II) within the evidence-based hierarchy behind SR's consisting of RCT's. SR's may also include meta-analyses, whereby the results from individual studies are collated to create a combined summary (pooled) of the effect estimate [219]. Conducting a meta-analysis can increase the power and provide a more precise estimate of the effect outcome. To prevent duplication and reporting bias of systematic reviews, a pre-registration of review protocols, for instance via the Prospective Register of Systematic Reviews (PROSPERO), exists where reviews and meta-analyses protocols are publicly registered [220]. As with clinical trials [221], guidelines also exist to facilitate the consistent reporting of systematic reviews and meta-analyses. In the case of systematic reviews and meta-analyses, the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) provides a checklist and flow-chart for authors to ensure uniformity and transparency in reporting SR's and meta-analyses. Notably, a new version of the PRISMA guidelines was released in 2020, however, at the time of the review included in this thesis [222] the 2009 guidelines were current [223], and therefore they have been used.

In this thesis, a SR and meta-analysis was performed to determine if vegetarian dietary patterns were associated with and/or able to modulate inflammation and/or immune biomarkers compared to those following non-vegetarian dietary patterns. Within the SR, studies from both observational cross-sectional studies and randomised controlled trials were

included and reviewed separately. This was due to the differences in study designs between observational and RCT's making comparison of outcomes more meaningful as intervention studies would assess the acute outcomes of vegetarian-based diets while observational studies would describe the longer-term effects on these diets. Additionally, by separating intervention and observational studies we were able to create a separation of the strength of the evidence which is recommended protocol to enable assessment on agreement or incongruity between the two types of evidence [224].

Each study within a SR is assessed for quality using published tools which can be used to inform the synthesis of the study findings and provide an overarching assessment about the body of evidence [219]. The most recognised and widely accepted tool for assessing bias in RCTs is the Cochrane Collaboration's risk-of-bias tool [219, 225]. As this tool is validated to assess RCTs, is transparent and descriptive in its output, and was been designed drawing on theory and evidence of the impact of bias on research findings[226] it was, therefore, applied to individual RCTs in this thesis to assess study quality. Due to the differences in the study methods between RCTs and non-RCTs, a different study quality tool was required for assessing the included non-randomised studies. For instance, in tools assessing RCTs quality questions are often asked around the blinding process, whereas in cross-sectional studies this is not relevant. The Newcastle-Ottawa Scale (NOS) is a widely utilised tool for assessing non-randomised trials with its content validity and inter-rater reliability established [227, 228]. A modified version of the NOS to complement the research question was, therefore, selected to assess the study quality in observational studies in this thesis.

While many strengths of SR's and meta-analyses exist, there are some drawbacks. Meta-analyses conducted across differing populations where disparate methods are used to assess

outcome variables have the potential to produce skewed results [219]. Publication bias may also skew results as it is more likely for studies to be published when they have significant or positive results compared to non-significant results [229], although, statistical methods can be applied to help to identify this limitation. In this thesis, funnel plots were generated and the Egger's test applied to assess for small study effects [230] and identify potential publication bias. Despite SR's and meta-analyses typically being the gold standard 'strength of evidence', if low quality studies are collated without acknowledgement a potentially misleading conclusion may be reached. To overcome this, assessment of the entire body of evidence should be performed which was completed in this thesis using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) method for both observational and intervention studies [35]. Most of these limitations can be addressed by adhering to standardised and transparent protocols outlined in the Cochrane Handbook for Systematic Reviews of Interventions [219] and reporting the systematic review according to the PRISMA statement, checklist and flow diagram [223] as occurred in this thesis.

2.6 Exercise trial

The National Health and Medical Research Council, Australia describes evidence obtained from a systematic review comprised of randomised controlled trials (RCTs) as the highest scientific evidence available, with well-designed individual RCTs ranking next [231]. RCTs provide high quality evidence due to their study design. Importantly, randomisation of the study participants prior to the study commencing largely reduces possible dissimilarities between groups which could confound the results [232]. Moreover, the inclusion of a carefully selected control group confirms any effects which may be observed in the intervention group by eliminating or minimising confounders such as familiarisation to the

study regime [233]. Dietary interventions are particularly prone to confounding and are, therefore, desirable to test the effects of foods or nutrients [234]. Despite RCTs being the gold standard, they have limitations. Namely, large scale RCTs are expensive to conduct and require an extensive time commitment [232, 235].

In the instance of this thesis, a cross-sectional study design, rather than a RCT was deemed to be suitable to examine the potential differences in exercise physiology between vegetarian and non-vegetarian dietary groups for several reasons. Firstly, a cross-sectional design provided a medium to examine habitual dietary consumption, likely to be more representative of intakes that endurance athletes following vegan dietary patterns were consuming in everyday life. Prescribing a vegan diet to participants may not provide an accurate representation of a vegan diet that the endurance athletes follow day to day. Secondly, for a RCT to address the aim of the study, the duration of the intervention would have had to be considerable to modulate many of the biomarkers of interest. For instance, changes in some serum inflammatory markers such as IL-6 and CRP can take several weeks to become physiologically apparent [236, 237] which would present challenges with regard to participant compliance and the feasibility of conducting such a study. Considering these points, a quasi-experimental, lab-based experimental design was selected to as the most appropriate study design.

2.7 Exercise testing

2.7.1 Biomarkers

Aerobic exercise can elicit a physiological response known as the acute phase response (APR) which is characterised by increased physiological strain and can be detected by examining relevant biomarkers [238]. Exercise induced inflammation can be desirable as it can assist with repair and recovery directly after exercise [239], whilst consistent exercise

promotes a systemic net positive anti-inflammatory effect. On the other hand, high-intensity exercise with insufficient recovery, induces a persistent dysregulation of the immune system and can increase the likelihood of illness [238]. For this reason, exploring inflammatory and immune markers in athletes is important. Exploration of this concept is justified as plant-based *foods* have been associated with lowered baseline inflammatory markers [240] and it is plausible that the differences between cytokine production during endurance performance may exist between individuals following vegetarian-based dietary patterns high in these food components.

Selecting appropriate cytokine markers to examine following exercise can be challenging as acute exercise bouts initiate complex inflammatory events and can be dependent on several variables including exercise duration, intensity, exercise type, familiarity of the exercise performed and participant age among others [127, 241, 242]. Of the acute phase response biomarkers investigated, some of the most researched pro-inflammatory biomarkers following exercise with sufficient detectability include tumour necrosis factor-alpha (TNF-α), IL-1β, IL-6 and CRP which are followed by the release of anti-inflammatory or regulatory cytokines including IL-4, IL-10, IL-1RA, and IL-13.

Interleukin-6 is one of the earliest and most prominent cytokines present following exercise, and up to 48 hours post exercise [243]. Interestingly, a major stimulus for increased IL-6 production is reduced muscle glycogen stores [104, 105]. Intakes of carbohydrates for those following vegetarian-based dietary patterns are typically higher than other dietary patterns and have been reported to increase muscle glycogen concentrations [106, 107]. Increases in IL-6 during exercise has also been reported to increase lipolysis [108], oxidation rate [108] and hepatic glucose production [109] making it a worthwhile marker to examine in this

thesis. Furthermore, IL-6 activation due to the exercise induced acute phase response has been suggested to assist in the adaptation of skeletal muscle to exercise, however, ongoing raised IL-6 can also increase inflammation and muscle wasting [244].

CRP production post exercise has had considerable research focus, likely due to the fact that it increases dramatically following exercise and remains elevated for a long period making it a recommended marker to use in exercise and inflammatory research [238]. For example, in some instances, CRP has been observed to increase 100 fold [245, 246] although, the exact zenith of CRP is varied in the literature with many variables influencing its production following exercise [238, 247]. In the context of exercise and the acute phase response, CRP has been proposed to play a role in eliminating damaged cells, attenuate the consequences of infection and tissue injury, suppress pro-inflammatory cytokines whilst inducing anti-inflammatory cytokines [248, 249]. IL-1, IL-10 and TNF-alpha can also be temporarily elevated at various time periods post prolonged exercise [250, 251] and have been suggested as useful markers to explore the inflammatory and immune processes following exercise. For this reason, these markers were selected as the key markers to examine in Study 6.

2.7.2 Exercise testing protocol

In this thesis, a range of physiology markers were examined. To reduce confounders between groups, a protocol was required where each participant acted as their own control. A modified version of Almada et al's protocol [252] was selected which includes an initial maximal aerobic capacity test on each participant to determine participants' relative work load for the exercise testing. Further, as this was a laboratory study rather than field study, other confounders apparent for exercise testing in maximal aerobic power and submaximal exercise could be tightly controlled with the laboratory temperature-controlled at 21-23 degrees

Celsius and a stable humidity (40-50%). Most importantly, it was selected as it has previously demonstrated elevated serum IL-6 following the protocol. The protocol also provided scope to examine a range of other relevant physiology markers of interest including heart rate, heart rate variability, muscle oxygen saturation and substrate utilisation at various varying intensities relative to each participant whilst minimising overall participant burden.

Implementing the Almada exercise testing protocol [252] has some limitations. Notably, whilst the protocol has successfully demonstrated increased cytokines following exercise, there are disparate findings in terms of the duration and intensity required to elevate the markers. There is some consensus that more intense exercise for a longer duration will elicit a greater APR response. Implementing a protocol of a long duration such as 24-48 hours with high intensity may bring with it other considerations such as ethical considerations pertaining to participant burden, fatigue and safety whilst also limiting some of the other physiology markers of interest. Further, participant burden and recruitment would be likely to be impeded. The Almada protocol, therefore, provides a good balance between participant burden, ethical considerations whilst still providing a platform to investigate all markers of interest.

2.8 Conclusion

In conclusion, this chapter has provided an overview of the methods, considerations and approaches used throughout this thesis to explore potential differences in dietary intake which may translate into differences in physiology between those following vegetarian and non-vegetarian-based dietary patterns. The thesis incorporates a range of study designs which have been justified as relevant to this thesis. The following chapters will address how they were performed and discuss the implications in respect to the thesis aims.

3 Examining dietary behaviours, motivations and supplementation use: An international survey of athletes following self-reported plant-based eating patterns | Study 1(a)

3.1 Introduction

As outlined in Chapter 1 vegetarian-based dietary patterns have been associated with protection against a range of diseases and conditions [12, 15, 28, 253-255]. However, motives for following vegetarian-based dietary patterns extend beyond the health benefits and include animal rights and environmental concerns [256]. More recently, the uptake of vegetarian-based dietary patterns among athlete populations has been gaining popularity, perhaps due to the perceived performance outcomes [57, 58]. To date, little research has been conducted into the motives of athletes following a dietary pattern of this nature or what these dietary patterns may look like. Understanding dietary behaviors and motives is important for individuals, health practitioners and researchers for several reasons. As Eustis et al [257] describe, examining motives can assist in identifying areas for dietary intervention, help create personalised approaches to dietary change, be utilised to illuminate differences in outcomes between dietary patterns for researchers and assist in tailoring public health nutrition messages.

Similarly, athletes following non-vegetarian dietary patterns often place an emphasis on nutritional components of foods and supplements known to support or enhance performance such as carbohydrates, protein and fluids and various sports specific supplements such as caffeine, creatine and nitrates [258] but little data exists exploring supplementation patterns in athletes following vegetarian-based dietary patterns. The aim of this study was therefore to explore the dietary behaviours including motivators and nutrition supplementation patterns in athletes following self-reported vegetarian-based eating patterns.

3.2 Methods

3.2.1 Survey development and design

A 52-item online survey was created to address the research aim. The survey was separated into several sections including respondent demographics, motives, physical activity, general eating patterns and supplementation use (**Appendix A**). Answers to all items were voluntary. Respondents were able to skip questions or choose non-responses, such as "unsure" or "not applicable". A validated physical activity tool (The International Physical Activity Questionnaire-Short Form IPAQ-SF)[259] was incorporated into the survey to gauge activity levels in respondents in addition to follow up questions exploring engagement in differing sports. Participants were excluded if they failed to complete the demographics section or reported having meat more than two times per week.

The survey was validated for construct (i.e. does the survey measure the theoretical construct it intends to) and face (i.e. is the survey suitable to achieve the aims) validity by a convenience sample of seven researchers with experience in conducting surveys. Briefly, the researchers rated each survey item for relevance, accuracy, clarity and appropriateness on a 4-point Likert scale as part of the Content Validity Index (CVI) [180]. The CVI for each item was calculated by dividing the number of raters who scored the item as 3 or 4 by the total number of raters (n=7). A score above 0·83 was considered adequate [260]. Additionally, each researcher was also asked to provide feedback regarding, ease of instructions, technical issues, time taken to complete the survey, or other information relevant to the improvement of the survey. The Checklist for Reporting Results of Internet E-Surveys (CHERRIES) was followed for the reporting of results for this survey [261]. Ethics approval was granted by the University of Wollongong Human Research Ethics Committee (2017/ 393).

3.2.2 Survey administration

The open-access online SurveyMonkey tool (SurveyMonkey, San Mateo, CA, U.S.A.; http://www.surveymonkey.com) was used to administer, collect and manage the data. An invitation to participate in the survey was posted to the ten largest social media Facebook groups by member size after searching; 'Vegetarian Athlete' and 'Vegan Athlete'. 'No Meat Athlete' Facebook groups were also invited individually to participate in the study. Tacit consent was provided by respondents completing the survey. Respondents were encouraged to share the details of the study to their own networks building upon Parkinson and Bromfield's research [177]. Prompts were provided via email at three time points with varied timing to increase the likelihood of responses rates and reduce non-response error. The survey was active from 30th May 2018 - 20th August 2018.

An additional external link to an Automated Self-Administered 24-hour (ASA24-AU) dietary recall was also included for those who were interested and formed the basis for Study 1b described in the next chapter (Chapter 4). Figure 3.1 outlines the flow for Study 1a and Study 1b.

3.2.3 Athlete status

Respondents were asked to self-identify with one of three athlete statuses

- recreational - participate to keep fit/healthy and for enjoyment
- competitive - participate to keep fit and healthy but have a somewhat structured training program with the aim to compete/win events and/or competitions
- professional – are paid to compete and/or have sponsors related to your sport.

3.2.4 Nutritional supplements

A series of questions were presented to the respondents related to three main categories including 'dietary/medicinal', 'muscle building' or improved energy' and 'performance

foods'. Respondents indicated if they had consumed the products or supplements over the past 12 months with the primary purpose to support or improve their performance. Respondents also had the opportunity to enter a free text response if they had consumed a supplement outside of those listed.

3.2.5 International Physical Activity Questionnaire-Short Form

Activity was defined in terms of Metabolic Equivalent Task (MET) scores whereby one MET is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 mL O_2/kg/min [262]. The results are presented as the estimation of energy expenditure in metabolic equivalent-minutes per week (MET-min/week). The IPAQ-SF questionnaires were administered online and processed in compliance with the 'Guidelines for data processing and analysis of the IPAQ-SF' [214]. Further details of the IPAQ-SF data processing and analysis are described in the statistical analysis (4.2.7)

3.2.6 Data management and statistical analysis

Data was analysed using IBM SPSS Statistics for Windows software (Version 26.0., 2017 Armonk, NY: IBM Corp.). Descriptive statistics summarised the characteristics of respondents and the aggregated responses to survey items. The IPAQ-SF data was cleaned in Microsoft Excel (Microsoft Excel, 2021 version 16.4) with total-MET minutes calculated in accordance with 'Guidelines for Data Processing and Analysis of the IPAQ short form' guidelines [263]. To determine vigorous activity minutes performed per week from the IPAQ-SF surveys, 'minutes of vigorous exercise per session' were multiplied by the 'number of sessions per week'. The same method was used to calculate moderate activity, and walking minutes per week. The summation of weekly minutes from vigorous, moderate and walking activity was used to calculate the MET-minutes using the following equations as stipulated in the IPAQ short form guidelines [264]. Walking MET-minutes/week = 3.3 * walking

minutes/week. Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes/week. Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes/week. The total weekly MET-minutes for each participant was then calculated by adding MET-minutes from each category. The Shapiro-wilks test was used to test normality. In order to compare the importance placed on different aspects of the diet between athlete status, participant responses were scored (extremely important = 5, very important = 4, important = 3, mildly important = 2, and not important = 1). Kruskal Wallace tests were then used to compare between athlete status. Where differences were found Mann Whitney-U tests were applied to determine where the differences existed between groups with Bonferroni correction applied. Significance was determined at $p<0.5$.

3.3 Results

3.3.1 Dietary outcomes

In total 921 respondents commenced the survey. Of these, 131 respondents exited the survey before completing the demographics section and were subsequently excluded from the analysis. A further nine respondents were excluded from the analysis after the exclusion criteria was applied (consumed meat at least 2 times/week) leaving a total of 781 respondents (**Figure 3.1**).

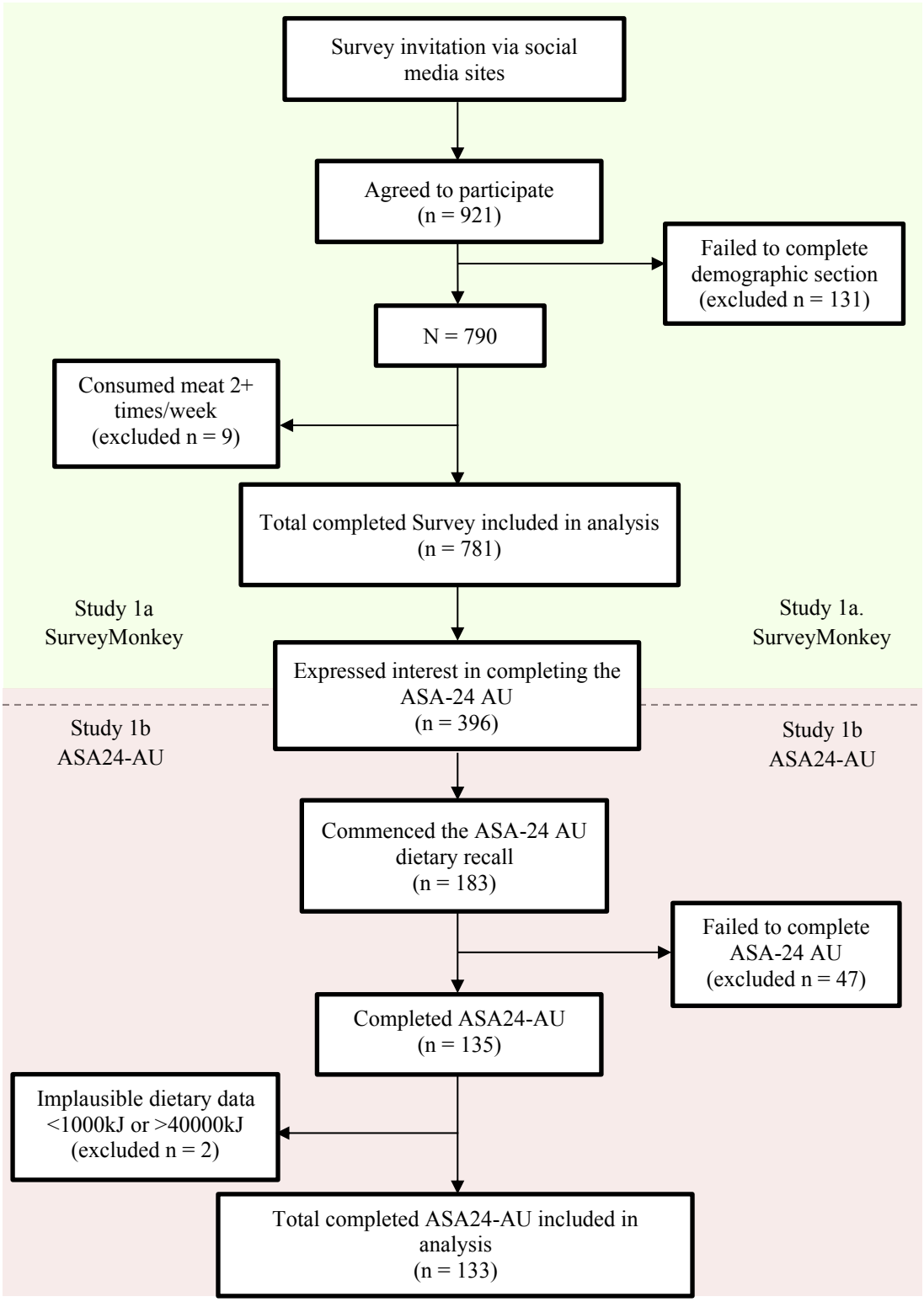


Figure 3.1. SurveyMonkey (Study 1a) and ASA24-AU (Study 1b) completion flowchart

3.3.2 Characteristics of respondents

Survey respondents tended to be female (72.3%), enrolled in, or completed a Bachelor's degree or higher (79.9%) and aged 20-49 years of age (86.9%). Respondents were predominately from the United States of America (25.0%), Australia (24.6%), or the United Kingdom (16.4%) with 10.9 % of respondents having completed formal nutrition training. Most respondents self-described as following a vegan (71.4%) or LOV (12.2%) eating pattern with a varied dietary duration (**Table** 3.1). There were 464 respondents who identified as recreational athletes, 198 as competitive athletes and 17 professional athletes. Total IPAQ MET-mins was 3814.5 (2346.0 - 6262.5 IQR) for the population. The most common forms of exercise were jogging/running (n=185), walking (n=94), weightlifting (n=76), cycling (n=76) and attending fitness classes (n=45) (**Figure** 3.2)

Table 3.1. Survey respondents' demographic and dietary behaviours (n=781)

Characteristics		**n (%)**
Gender	Female	565 (72.3)
	Male	207 (26.5)
	Prefer not to disclose	2 (0.3)
	Prefer to self-describe	5 (0.6)
	Missing	2 (0.3)
Age (years)	18-19	37 (4.7)
	20-29	300 (38.4)
	30-39	243 (31.1)
	40-49	136 (17.4)
	50-59	51 (6.5)
	60+	14 (1.8)
Region of birth	United States of America	195 (25.0)
	Australia	192 (24.6)
	United Kingdom	128 (16.4)
	Europe	61 (7.8)
	Canada	53 (6.8)
	New Zealand	20 (2.6)
	South Africa	19 (2.4)
	Scandinavia	14 (1.8)
	India	11 (1.4)
	Other	88 (11.3)
Education	Secondary School	60 (7.7)
	Diploma or Equivalent	94 (120)
	University (Undergraduate)	329 (42.1)
	University (Honours/Masters)	221 (28.3)
	Doctorate	74 (9.5)
	Other	1 (0.1)
	Missing	2 (0.3)
Formal nutrition training	No	694 (88.9)
	Yes	85 (10.9)
	Missing	2 (0.3)
Self-described eating pattern	Vegan	558 (71.4)
	Pescetarian	33 (4.2)
	Lacto-vegetarian	34 (4.4)
	Ovo-Lacto-vegetarian	95 (12.2)
	Ovo-vegetarian	22 (2.8)
	WFPB	14 (1.8)
	Other	23 (2.9)
	Missing	2 (0.3)
Diet duration	0-6 Months	42 (5.4)
	6-12 Months	76 (9.7)
	1-2 Years	125 (16.0)
	2-5 Years	256 (32.8)
	5-10 Years	145 (18.6)
	10-20 Years	73 (9.3)
	20+ Years	61 (7.8)
	Unsure	1 (0.1)
	Missing	2 (0.3)

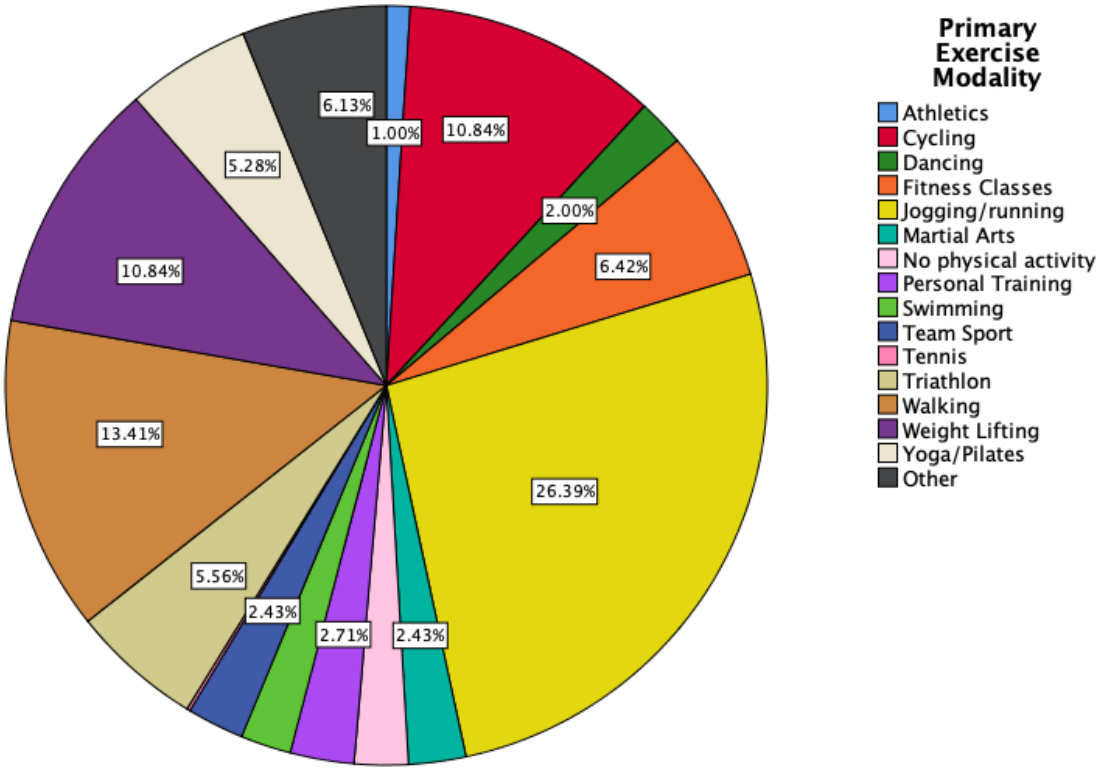


Figure 3.2. Primary exercise modality in respondents

3.3.3 Dietary behaviours, perceptions and motivations

Participant's perceptions of diet for exercise varied considerably. Diet was considered extremely important for hydration (27%) endurance capacity (17.6%) and decreasing body weight (10.4%). On the other hand, dietary intake was not considered important for increasing body weight (53.4%), decreasing delayed onset muscle soreness (30.7%) or muscle fatigue (25.7%) **Table** 3.2**.** Principal motivation for respondents adhering to a vegetarian-based dietary pattern included animal rights (87.5%), environmental concerns (75.8%), health reasons (70.0%). Out of 774 respondents, 364 (47%) reported including specific foods to increase their physical performance. The foods described were varied but were self-identified as: high protein whole foods, protein powders, leafy green vegetables, nuts and seeds, fruit and increased carbohydrates before, after or during heavy training periods or races **(Table 3.3**). Further, 291 (37.5%) respondents reported restricting or eliminating certain foods to increase their physical activity. The majority of foods restricted or eliminated included "processed foods" or "junk foods" with oils/fats, added sugars and alcohol typically mentioned (**Table 3.3**). From the 178 respondents who reported following a specific macronutrient dietary pattern high carbohydrate, high protein and low fat were most often adopted.

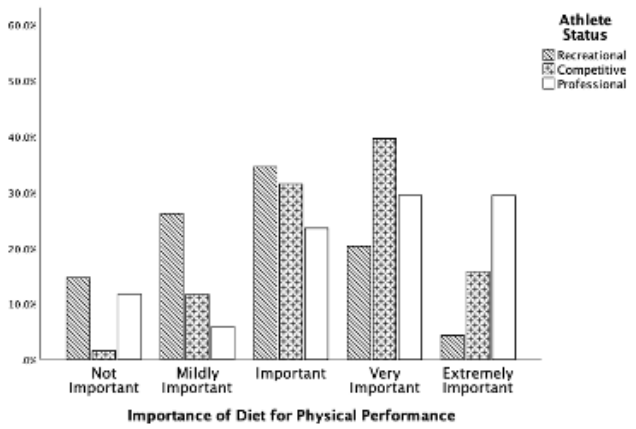
Table 3.2. Total survey respondents' perceptions of the importance of diet for exercise performance and body composition outcomes (n=777).

Importance of diet for	Not Important n %	Mildly Important n %	Important n %	Very Important n %	Extremely Important n %
Hydration	38 (4.9)	61 (7.9)	181 (23.4)	283 (36.6)	211 (27.3)
Endurance Capacity	120 (15.4)	102 (13.1)	222 (28.6)	196 (25.2)	137 (17.6)
Decreasing Body Weight	188 (24.2)	186 (23.9)	194 (25.0)	128 (16.5)	81 (10.4)
Recovery	105 (13.5)	160 (20.6)	242 (31.1)	191 (24.6)	79 (10.2)
Muscular Strength	153 (19.7)	146 (18.8)	251 (32.3)	156 (20.1)	70 (9.0)
Muscular Power	173 (22.3)	145 (18.7)	237 (30.5)	156 (20.1)	66 (8.5)
Physical Performance	96 (12.4)	169 (21.8)	262 (33.7)	189 (24.3)	61 (7.9)
Body Fat	123 (15.9)	196 (25.3)	235 (30.3)	164 (21.2)	57 (7.4)
Body Composition	137 (17.7)	175 (22.6)	266 (34.3)	142 (18.3)	56 (7.2)
Muscle Fatigue	199 (25.7)	157 (20.3)	216 (27.9)	147 (19.0)	56 (7.2)
DOMS	238 (30.7)	177 (22.8)	194 (25.0)	121 (15.6)	45 (5.8)
Increasing Body Weight	415 (53.4)	102 (13.1)	126 (16.2)	91 (11.7)	43 (5.5)

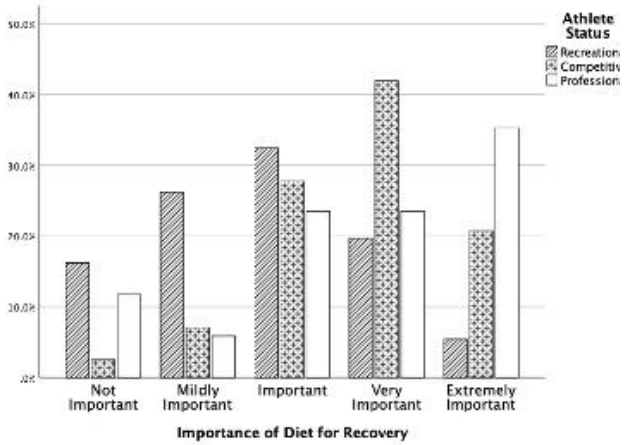
DOMS: Delayed Onset Muscle Soreness

Table 3.3. Survey respondents' dietary behaviours, motives and characteristics (n = 777).

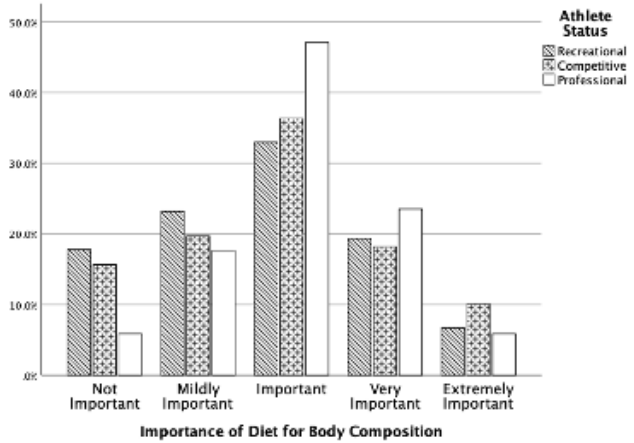
	No n (%)	Yes n (%)
Restrict Energy to Increase Physical Performance	658 (84.8)	118 (15.2)
Include Foods to Increase Physical Performance	410 (53.0)	364 (47.0)
Restrict or Eliminate Foods to Increase Physical Performance	485 (62.5)	291 (37.5)
Follow a Specific Macronutrient Diet	601 (77.2)	178 (22.8)
High Carbohydrate	707 (90.8)	72 (9.2)
Low Carbohydrate	753 (96.7)	26 (3.3)
High Protein	736 (94.5)	43 (5.5)
Low Protein	775 (99.5)	4 (0.5)
High Fat	766 (98.3)	13 (1.7)
Low Fat	708 (90.9)	71 (9.1)
Reasons for following plant-based diet		
Family/Friends	777 (99.7)	2 (0.3)
Religion/Spiritual	715 (91.8)	64 (8.2)
Always eaten this way	754 (96.8)	25 (3.2)
Improve Physical Performance	592 (76.0)	187 (24.0)
Environmental Concerns	192 (24.6)	587 (75.4)
Animal Rights	103 (13.2)	676 (86.8)
Weight Loss	676 (86.8)	103 (13.2)
Health	237 (30.4)	542 (69.6)


Athlete Status
Recreational
Competitive
Professional
Not Important
Mildly Important
Important
Very Important
Extremely Important
Importance of Diet for Physical Performance

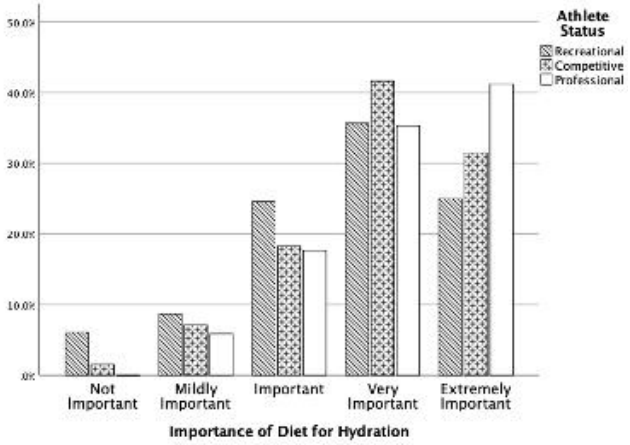
a)


Athlete Status
Not Important
Mildly Important
Important
Very Important
Extremely Important
Importance of Diet for Recovery

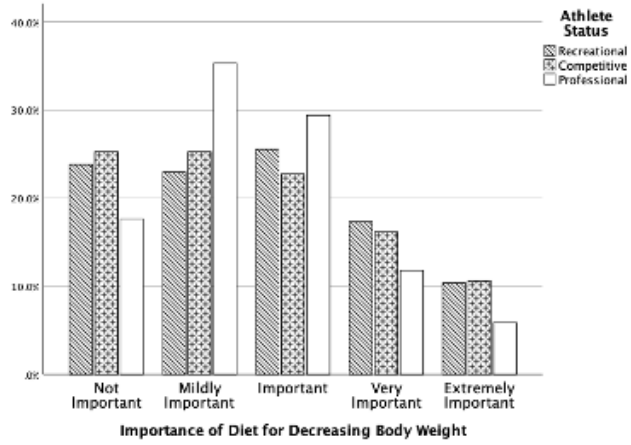
b)


Athlete Status
Recreational
Competitive
Professional
Not Important
Mildly Important
Important
Very Important
Extremely Important
Importance of Diet for Body Composition

c)


Athlete Status
Recreational
Competitive
Professional
Not Important
Mildly Important
Important
Very Important
Extremely Important
Importance of Diet for Hydration

d)


Athlete Status
Recreational
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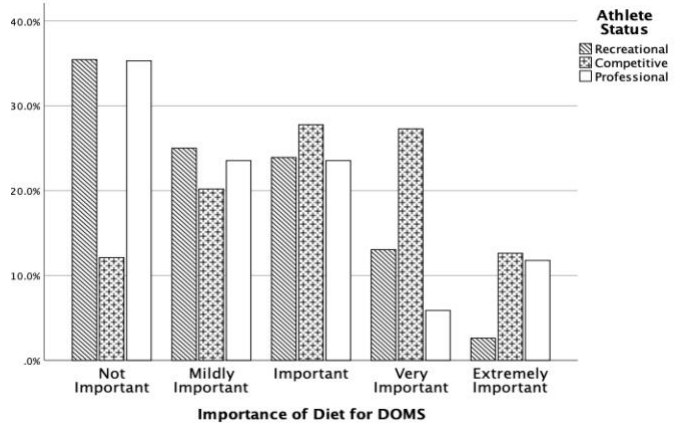
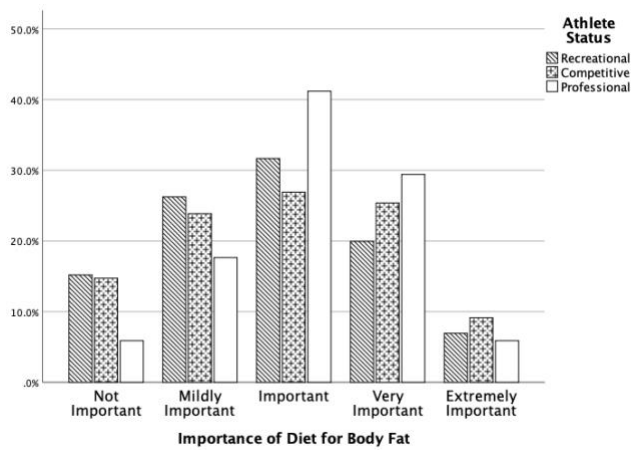
Not Important
Mildly Important
Important
Very Important
Extremely Important
Importance of Diet for Decreasing Body Weight

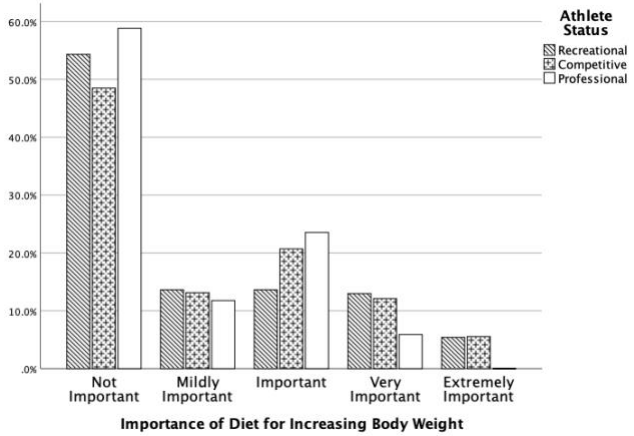
e)


Athlete Status
Recreational
Competitive
Professional
Not Important
Mildly Important
Important
Very Important
Extremely Important
Importance of Diet for DOMS

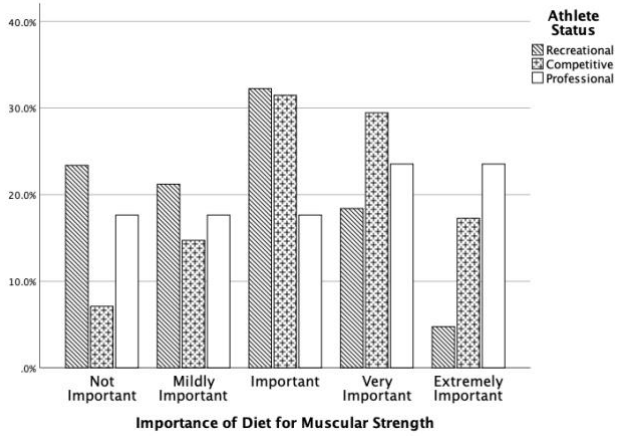
f)



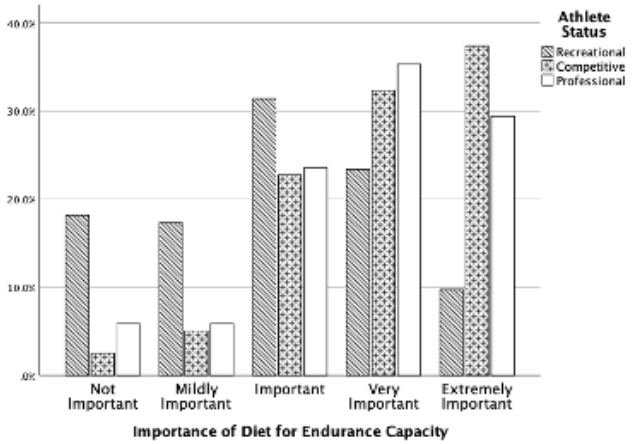
g)



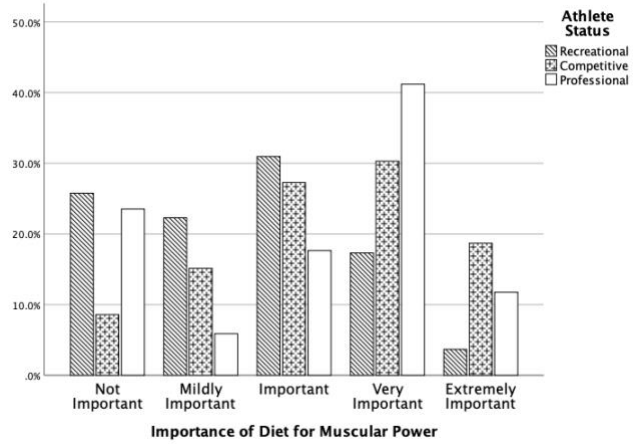
h)



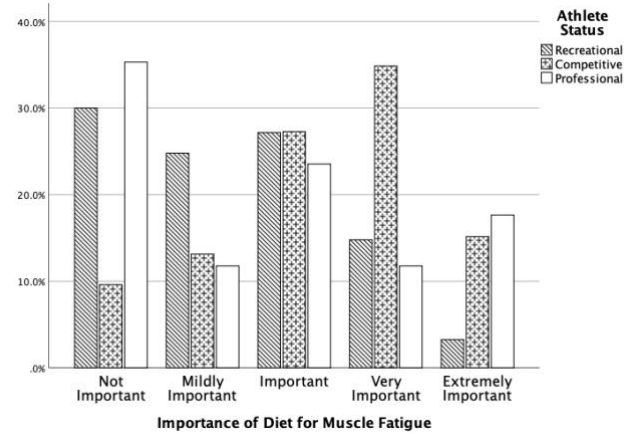
i)



j)



k)



l)

Figure 3.3. Importance of diet for various components of performance by athlete status (a-l).

Figure 3.3 depicts the perceived importance of various dietary components by athlete status (recreational, competitive and professional). A decreasing stepwise trend is observed with professional athletes perceiving diet for physical performance (**Fig. 3.3.a**), recovery (**Fig. 3.3.b**), hydration (**Fig. 3.3.d**), muscular strength (**Fig. 3.3.i**) and muscular fatigue (**Fig. 3.3.l**) as 'extremely important' compared to competitive athletes, who again place more importance on the above components than recreational athletes. The majority of respondents perceived the importance of diet for hydration to be either important, very important or extremely important across all three athlete groups (**Fig 3.3.d**). On the other hand, diet for increasing body weight was perceived to be not important with by approximately 50% of respondents across all athlete groups (**Fig 3.3.h**).

When numerical scores were assigned to athlete perceptions of components of dietary importance to allow comparison between athlete status, it was found that recreational athletes placed significantly less importance on diet for; preparing for physical performance, recovery, muscular power and endurance capacity compared to professional and competitive athletes ($p<0.05$). Competitive athletes placed significantly more emphasis on diet for hydration, delayed onset muscle soreness, muscular fatigue and muscular strength compared to professional or amateur athletes ($p<0.5$). No significant differences between athlete status and importance of diet for 'increasing body weight', 'decreasing body weight' or 'body composition' were observed.

After participants were asked to record the three most likely sources to obtain their nutritional information from, it was observed that internet searches (62.2%) and academic journals (25.2%) were most heavily relied upon whilst coaches (5.8%) and teammates (6%) were the least likely (**Figure** 3.4).

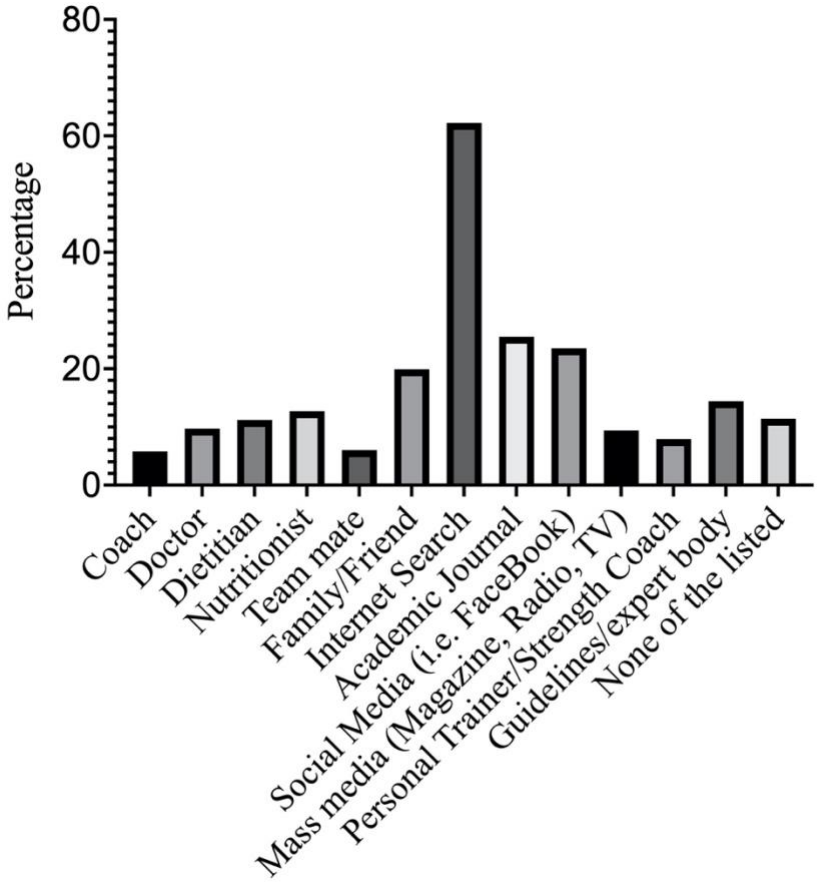


Figure 3.4. Most commonly relied upon sources for obtaining nutritional information by (percentage of respondents).

3.3.5 Supplement and nutrition product use

Vitamin B12 was the most commonly reported supplement with 58.1% of respondents indicating that they consumed this supplement in the past year. The next highest reported supplements were protein powder (36.3%) and vitamin D (35.9%). BCAA (6.1%), creatine (6.3%) and energy drinks (6.7%) were the least consumed products over the past year. Supplements frequently entered as free text included magnesium (4.3%), turmeric (1.0%) and beta-alanine (0.7%) (

Table 3.4**).**

Table 3.4. Survey respondents' supplement and nutrition product consumption over past 12 months (n=774).

	No	**Yes**	**Unsure**
	n	n	n
Sports specific supplement			
Protein powder	492 (63.6%)	281 (36.3%)	1 (0.1%)
Caffeine	533 (68.9%)	239 (30.9%)	2 (0.3%)
Electrolytes	629 (81.3%)	144 (18.6%)	1 (0.1%)
Protein bar	650 (84.0%)	123 (15.9%)	1 (0.1%)
Sports gels	661 (85.4%)	112 (14.5%)	1 (0.1%)
Sports drinks	684 (88.4%)	88 (11.4%)	2 (0.3%)
Energy drinks	722 (93.3%)	52 (6.7%)	0 (0.0%)
BCAA	722 (93.3%)	47 (6.1%)	5 (0.6%)
Creatine	665 (93.7%)	45 (6.3%)	0 (0.0%)
CHO sports bar	736 (95.1%)	37 (4.8%)	1 (0.1%)
Bicarbonate soda	764 (98.7%)	10 (1.3%)	0 (0.0%)
Traditional nutritional supplement			
Vitamin B12	324 (41.9%)	450 (58.1%)	0 (0.0%)
Vitamin D	496 (64.1%)	278 (35.9%)	0 (0.0%)
Iron	587 (75.8%)	187 (24.2%)	0 (0.0%)
Multi-vitamins	588 (76.0%)	186 (24.0%)	0 (0.0%)
Probiotics	623 (80.5%)	149 (19.3%)	2 (0.3%)
Vitamin C	645 (83.3%)	129 (16.7%)	0 (0.0%)
EPA/DHA	649 (83.9%)	123 (15.9%)	2 (0.3%)
B complex	669 (86.4%)	105 (13.6%)	0 (0.0%)
Zinc	683 (88.2%)	91 (11.8%)	0 (0.0%)
Calcium	691 (89.3%)	83 (10.7%)	0 (0.0%)
Iodine	727 (93.9%)	47 (6.1%)	0 (0.0%)
Alpha-LA	734 (94.8%)	38 (4.9%)	2 (0.3%)
Vitamin E	742 (95.9%)	32 (4.1%)	0 (0.0%)

EPA/DHA; eicosapentaenoic acid/docosahexaenoic acid, BCAA: Branched-chain amino acids, Alpha-LA: Alpha-linolenic acid, CHO sports bar: Carbohydrate sports bar.

3.4 Discussion

This study has provided an overview of dietary behaviors, motivations for following a vegetarian-based diet and a detailed exploration of supplementation patterns in an under-

researched population of athletes following plant-based dietary patterns in a large cohort and offers some important findings. Firstly, this study has shown that among athletes following vegetarian-based dietary patterns a considerable proportion do so for the perceived exercise performance benefits. Secondly, sports supplements with known and accepted ergogenic effects were seldomly reported to be used and could be an area for consideration for athletes following vegetarian-based diets.

3.4.1 Dietary behaviors, perceptions, and motivations

Historically, there have been two primary motivations for adopting and maintaining a vegetarian-based dietary pattern including animal-orientated concerns and perceived health outcomes [162, 265, 266]. In this cohort of self-reported athletes reportedly following a vegetarian-based dietary pattern these two motives were also highly represented although, interestingly the second most cited motive for following a vegetarian diet was for environmental preservation (75.8%), in contrast to animal orientated concerns (87.3%) and health (70.0%). Perceived impact on exercise performance and weight loss were also heavily reported with 24.1% and 13.3% of respondents reporting these motivations respectively. These results suggest a changing pattern of motivators for following vegetarian-based diets, with greater emphasis on environmental concerns and improved performance.

Documentaries such as ‘The Game Changers’ which promotes the potential benefits of a vegetarian-based diet for athletes has attracted extensive media attention [267] and may contribute to the number of athletes adopting a vegetarian-based diet. Similarly, popular documentaries such as ‘Cowspiracy’ outlining the environmental impact of meat production can have a substantial influence on consumer behaviour which may be shaping changes in vegetarian-based dietary motives in both athletes and non-athletes [268]. Another explanation

may be that previous research has 'siloed' vegetarian-based dietary motivations which likely disregards the multifactorial nature of adoption of plant-based dietary patterns [269]. For example, often there are multiple reasons for ones decision to follow a vegetarian-based diet which was observed in this study. Allowing participants to select only one motive for adherence to the diet may not fully encapsulate the complexities of dietary motivations. Multiple responses for dietary motivation in this study was purposefully incorporated to capture the multifactorial motives more holistically for dietary pattern selection and should be included in future research exploring this topic.

This study has shown that in athletes following a vegetarian-based diets new motives outside the traditional two (health, animal rights) such as weight loss and increasing performance are emerging as considerable motivations for adoption of this dietary pattern. As more athletes adopt vegetarian-based dietary patterns, understanding these motives becomes increasingly important as there may be a flow on health implications for the individual athletes. Further, understanding these motivations and dietary patterns can be used to tailor public health nutrition messages for athletes following this diet. Diet quality in these athletes should be first examined to determine what, if any nutritional and/or health implications might exist and how the dietary pattern may influence performance outcomes. This information will be useful to inform the health message which may help guide dietetic practice and will be explored further in Study 1b.

As this study incorporated a range of athlete levels and exercise modalities it is somewhat difficult to meaningfully contrast the dietary behaviours and perceptions in this study, to others. This is because exercise related dietary behaviours are complex and vary according to sex, sporting modality, an athlete's competition level, and training volume and intensities

[270, 271]. Nonetheless, the present study has illuminated some general dietary behaviours in this population group of athletes following vegetarian-based dietary patterns. In this group of mixed self-reported recreational, competitive and professional athletes 47% reported that they include foods to improve performance in some way which appears to align with other research in non-vegetarian populations [270]. The importance of diet for various components of performance in the present study were observed to differ between athlete status (recreational, competitive and professional) in those following vegetarian-based diets. This finding is in agreement with other research showing that athlete caliber influences dietary behaviours and perceptions [271, 272].

Some interesting findings emerged regarding where the respondents were most likely to obtain their nutritional information from (**Fig 3.4**). An 'internet search' was the most common place of obtaining nutritional information in this cohort, which aligns with other research [273]. However, in contrast to other studies where reliance on dietitians, nutritionists and sports coaches were all in the top reported sources of obtaining nutrition information [270, 273, 274], the results in this cohort do not reflect this. Perhaps there is an underlining concern within athletes who follow vegetarian-dietary patterns that the wider medical and dietetic community will not accept their dietary choices, particularly for those following vegan diets which can have historically been associated in a negative context by society [275]. Another plausible explanation may be simply that different sporting modalities and athlete levels may influence access and need to consult nutrition professionals. This should be explored in more depth as a considerable proportion of respondents reported consulting unreliable sources in this cohort (23.5% social media and 19.9% family or friend).

3.4.2 Supplementation

Sports supplements are increasingly being used by all types of athletes yet the science has

struggled to document their safety, quality and effectiveness[276, 277]. Athletes seldomly seek education from reputable sources on the use of sports supplements leaving them vulnerable to misinformation [277]. This could subsequently result in health problems, substandard athletic performance and potentially even 'inadvertent doping' due to the ingredients used in some supplements [278, 279]. Furthermore, the ergonomic effects of many sports supplements is controversial thereby rendering them an important topic for consideration and exploration.

Interestingly, performance supplements with known ergogenic effects with strong scientific underpinnings (Sodium bicarbonate, beta-alanine, creatine, dietary nitrate, glycerol and caffeine) [280] were seldomly consumed. Caffeine consumption was the highest of these supplements consumed reported in 30.9% of the respondents which is similar to non-vegetarian athletes in other cohorts [272]. Caffeine is recognised to provide ergogenic effects via a range of mediums [281] and its moderate to high usage in this cohort appears warranted.

Consistent ergogenic benefits of creatine supplementation have been documented in the literature [282]. Creatine is made endogenously at a rate of approximately 1g/day, although creatine requirements have been suggested to be up to 2g/day for an average 70kg male [283]. Additional creatine can be acquired exogenously via dietary consumption, although only foods of animal origin contain creatine leading those following vegetarian-based diets to exhibit lower muscle total creatine stores [282]. The lower muscle creatine concentrations has led some to speculate that those following un-supplemented vegetarian-based diets may be at a performance disadvantage, particularly during anaerobic exercise when compared to those consuming un-supplemented omnivorous diets. The body of evidence does not currently support this notion however [284]. Further, as athletes following vegetarian-based diets have

lower creatine stores, it has been suggested that vegetarians may benefit from creatine supplementation to a greater extent than those supplementing creatine and also consuming animal products but this is also not supported by the balance of evidence [284, 285]. Creatine consumption in this study appears similar or marginally lower to non-vegetarian populations, but usage tends to vary with sporting type and athlete status (elite vs amateur for example) [272, 286, 287]. The use of creatine as an ergogenic supplement has been found to be effective mainly in high-intensity sports or resistance training by augmenting the rate of phosphocreatine resynthesis [288], but may also improve aerobic capacity via greater shuffling of ATP from mitochondria [282]. As creatine supplementation trials have reliably demonstrated ergogenic outcomes for both omnivorous and vegetarian-based athletes, creatine supplementation could be considered for the serious athlete looking to optimise performance.

Only 450 (58.1%) respondents reported using a vitamin B12 supplement in the past 12 months despite this being the recommendation for those following a vegan diet [44]. This rate was consistent with other research with 52% of endurance runners following vegan diets reporting to supplement vitamin B12 [289]. Although, in the present study the survey question asked about supplementation for performance outcomes ("*This section asks you about any supplements you currently consume to maximise your athletic performance"*) and vitamin B12 is not typically consumed for performance outcomes which may have influenced the response outcome. Compared to athletes following vegan dietary patterns, those athletes following non-vegan diets appear to supplement vitamin B12 far less (19%) [290]. Regardless, both athletes and non-athletes following vegetarian-based, and in particular vegan diets, must regularly consume foods fortified with vitamin B-12 or specifically supplement the vitamin, as few plant sources contain traces of vitamin B-12 and long-term

adherence to a vegetarian-based diet can result in a deficiency with considerable repercussions [44, 291].

The low rates of supplemented docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are interesting as vegetarian-based population groups are known to have lowered levels of long-chain polyunsaturated omega-3 fatty acids (LC n-3 PUFA) compared to groups who consume fish and marine products [124]. Consumption of dietary LC n-3 PUFA's are acknowledged to blunt the physiological stress associated with strenuous exercise [292] whilst DHA can be incorporated into the membranes of cardiac and skeletal muscle fibres [293, 294] likely improving skeletal muscle contractile efficiency [295]. Furthermore, LC n-3 PUFA's are recognised to provide a range of health benefits none more so than improving cardiovascular function [296-299].

3.5 Limitations and Strengths

Whilst this study was the first to provide an insight into dietary behaviours in athletes self-reporting to follow plant-based dietary patterns, there were some limitations. The respondents engaged in an array of sports and physical activity modalities and were from a large range of countries. The generalisability of the results from this study is therefore limited. Finally, the data obtained via this survey was self-reported and may therefore lack of representativity and may be prone to biased estimates [300], especially with identifying suitable dietary patterns and athlete status. There are also substantial strengths to this study. Research examining dietary behaviours and nutrient status in athletes following vegetarian-based dietary pattern is scarce and this study has considerably contributed to the body of literature. With 924 respondents recruited this study has provided one of the largest self-reported vegetarian-based athletic populations in the literature and has explored nutrient status and diet quality in

depth.

3.6 Conclusion

To our knowledge this is the first study to explore dietary behaviours, motives and supplementation patterns in self-reported athletes following vegetarian-based diets and has provided some key insights. The current study has provided evidence that while predominant motivators for following this diet were concerns regarding environmental impact and animal welfare, approximately one quarter of athletes surveyed were adopting a diet of this nature with the aspiration of improving their exercise performance. Vegetarian-based dietary patterns have been deemed appropriate for all levels of activity, and this study provides preliminary evidence that within athletic populations there may be a desire to improve performance using a vegetarian-dietary pattern. However, empirical evidence examining diet quality and nutrient status in athletes is scarce, requiring further investigation.

4 Diet quality and nutrient status in an international survey of athletes following a self-reported vegetarian-based eating pattern | Study 1(b)

4.1 Introduction

Well-planned vegetarian-based diets, including vegan diets, have been deemed appropriate for all levels of physical activity [44] with preliminary evidence showing no differences, at least acutely, between muscular power, muscular strength, aerobic or anaerobic performance outcomes in those following vegetarian and non-vegetarian based eating patterns observed to date [62]. Although, some researchers suggest that athletes who are following plant-based eating patterns may find it harder to obtain certain nutrients such as calcium, vitamin B12, vitamin D, iodine, and iron [43].

Dietary components such as these are undoubtedly important for an athlete's training and competition, and can also influence the overall quality of an athlete's diet [301] which is essential for optimal health and indicative of nutrient adequacy [302]. As noted in Chapter 2, diet quality indexes reflect a standard of 'healthfulness' related to a pattern of dietary intake [201] and may be of particular interest for athletes following vegetarian-based diets as they inherently eliminate some of the traditional food groups with little evidence currently examining this topic. As athletes often focus on certain nutritional components to improve their performance, they may overlook others, thereby impeding their overall diet quality. Similarly, athletes following vegetarian-based diets may eliminate or substitute traditional food groups impacting the healthfulness of their dietary pattern which may have long-term health repercussions. Many methods of assessing diet quality exist, two of which include the Alternate Healthy Eating Index (AHEI-2010) [303] and the Dietary Phytochemical Index (DPI) [213] which have been described in detail in chapter 2.

Study 1a provided an overview of dietary behaviors, motivators and supplementation patterns in a population of athletes following plant-based dietary patterns, however empirical data

concerning the nutrient intake status and diet quality of athletes following vegetarian diets, is limited. It was therefore the aim of this study to assess the nutrient status and overall diet quality of athletes self-reporting to follow a vegetarian-based dietary pattern.

4.2 Methods

4.3 Participants and dietary intake collection

Participants who completed the online survey (outlined in chapter 3) were asked if they would like to partake in an additional research component exploring their nutritional status. Interested respondents were sent an external link to an Automated Self-Administered 24-hour (ASA24-AU) recall. The ASA24-AU is a web-based tool using a validated methodology for the collection of dietary intake over the previous 24 hours [191]. Intake of dietary energy, macro- and micronutrients and supplements reported in the ASA24-AU was reported using AUSNUT 2011-13 [304].

4.3.1 Diet quality assessment

4.3.1.1 *AHEI-2010*

A modified version of the AHEI-2010 was implemented, whereby the methodology described by Wang et al [305] was applied due to the dietary data being collected via an 24 hour recall (ASA-AU-24). A modification from the original AHEI-2010 was made to align the scoring with the Australian Dietary Guidelines [306] with weights converted from imperial to metric. To determine the scoring of the 11 components, an a priori scoring framework was created using the Australian Health Survey ADG database [307]. Food items were converted to either serves per 100g (vegetables, fruits, wholegrains, sweetened sugar sweetened beverages, nuts and legumes, red/processed meats), kJ per 100g (trans fat and PUFA), mg per 100g (EPA + DHA) or drinks per 100g (alcohol). Each food consumed from the ASA24-AU was then

matched to the database using the 8-digit survey ID codes. Each component was scored on a 0 - 10-point scale and subsequently summed to obtain the total score. Nondrinkers received a score of 2.5. Details of the scoring calculation are outlined in **Table** 4.1**.**

Table 4.1. Scoring criteria for minimum and maximum scores for the AHEI.

Component	Criteria for minimum score (0)	Criteria for maximum score (10)
Vegetables (servings/day)	0	≥5
Fruit (servings/day)	0	≥4
Whole grains (g/day)		
Women	0	75
Men	0	90
Sugar-sweetened beverages and fruit juice (servings/day)	≥1	0
Nuts and legumes (servings/day)	0	≥1
Red/processed meat servings/day	≥1.5	0
Trans fat (% of energy)	≥4	≤0.5
Long-chain omega-3 fatty acids (DHA/EPA; mg/day)	0	250
PUFA (% of energy)	≤2	≥10
Sodium (mg/day)*	Highest decile	Lowest decile
Alcohol(drinks/day)		
Women	≥2.5	0.5-1.5
Men	≥3.5	0.5-2.0
Total	0	110

4.3.1.2 *Dietary Phytochemical Index*

As described in chapter 2, The DPI provides a percentage of dietary calories supplied by foods typically high in phytochemicals including: fruits, vegetables, legumes, nuts, seeds and whole grains. To calculate the DPI, the AUSNUT 2011-13 5-digit food group codes of all foods reported by survey participants were reviewed by an Accredited Practising Dietitian (APD) to determine if the foods were high or low in phytochemicals complying with previous phytochemical classifications [213, 308]. The food groups deemed to be high in phytochemicals as per McCarty's framework [213] included vegetables, fruits, whole grains, legumes, nuts and seeds, fruit and vegetable juices, soy products and wine. Added oils, refined sugars, refined grains, heavily processed foods, potato products, beer, juices from concentrates, hard liquors, and animal products were deemed to be low in phytochemicals. When required, the AUSNUT 2011-13 food recipe file [309] and food detail file [304] were examined to gain a deeper insight into the food group phytochemical status when it was unclear. A second APD reviewed each 5-digit food group for accuracy. The dietary photochemical index (DPI) was calculated from the ASA24-AU tool and expressed as the percentage of dietary energy derived from the foods rich in phytochemicals [213].

4.3.2 Data management and statistical analysis

Data were analysed using IBM SPSS Statistics for Windows software (Version 26.0., 2017 Armonk, NY: IBM Corp.). Responses where ASA24-AU records were only partially completed were excluded to improve the quality of the data, as partially/incomplete results would reduce the accuracy of the dietary outcome measures. The Shapiro-wilks test was used to test normality. Correlations were explored using Pearson's or Spearman's tests depending on normality between demographics, dietary motives, specific macronutrient adherence, total MET minutes, time spent sitting and DPI and AHEI-2010. The Kruskal Wallace and Mann

Whitney-U tests were used to determine if differences existed between the nutrient intakes and diet quality and self-reported athlete status, diet duration, education level, formal nutrition training and nutrient profiles with Bonferroni correction applied. Significance was determined at $p<0.5$.

4.4 Results

4.4.1 Participants

From the survey responses (Chapter 3), 396 respondents expressed interest in completing the ASA-24 AU, 183 commenced the ASA24-AU dietary recall and 136 finished the tool to completion. Two respondents had implausible dietary data either exceeding 40,000kJ or falling below 1000kJ for the total daily energy intake and were subsequently excluded. A total of 133 respondents completed the ASA24-AU dietary recall and were included in the nutrient analysis. Participant characteristics are described in **Table 4.2. Participant characteristics from ASA24-AU**

Table 4.2. Participant characteristics from ASA24-AU

Characteristics		**n (%)**
Sex	Female	94 (70.7)
	Male	39 (29.3)
Age (years)	18-19	1 (0.8)
	20-29	45 (33.8)
	30-39	46 (34.6)
	40-49	33 (24.8)
	50-59	6 (6.5)
	60+	2 (1.5)
Education	Secondary School	6 (4.5)
	Diploma or Equivalent	13 (9.8)
	University (Undergraduate)	46 (34.6)
	University (Honours/Masters)	45 (33.8)
	Doctorate	23 (17.3)
	Missing	2 (0.3)
Formal nutrition training	No	111 (83.5)
	Yes	22 (16.5)
Self-described eating pattern	Vegan	84 (63.2)
	Pescetarian	7 (5.3)
	Lacto-vegetarian	9 (6.8)
	Ovo-Lacto-vegetarian	23 (17.3)
	Ovo-vegetarian	3 (2.3)
	WFPB	4 (3.0)
	Other	3 (2.3)
Diet duration	0-6 Months	7 (5.3)
	6-12 Months	12 (9.0)
	1-2 Years	26 (19.5)
	2-5 Years	41 (30.8)
	5-10 Years	25 (18.8)
	10-20 Years	7 (5.3)
	20+ Years	15 (11.3)
Athlete Status	Recreational	83 (62.4)
	Competitive	45 (33.8)
	Professional	3 (2.3)
	Missing	2 (1.5)
	Mean	**S.D**
BMI	22.99	3.22
MET-Minutes (Weekly total)	4663.74	3052.96
Sitting minutes (Daily total)	663.9	159.49

BMI, Body Mass Index, MET-Minutes, Metabolic equivalent minutes, WFPB, Wholefood plant-based.

4.4.2 Nutrient Status and Diet quality

Of the 133 respondents who completed the ASA-24-AU, their self-identified dietary pattern was as follows: vegan (n=84), pescatarian (n=7), lacto-vegetarian (n=9), lacto-ovo-vegetarian (n=23), ovo-vegetarian (n=3), WFPB (n=4) and other (n=3). Energy intake was 8897.3 kJ/day (IQR 6982.1 - 11820.4; **Table** 4.3). The absolute median protein intake was 72.0 grams/day (IQR 56.4 - 97.2) whilst the relative median protein intake was 1.18 grams/kg/day (IQR 0.832-1.52). Carbohydrate intake was 235.5g/day (IQR 182.4 - 316.8) with the median relative intake 3.6g/kg (IQR 2.9 - 5.0) whilst total fat intake was 82.7 (IQR 55.5 - 115.5) grams/day. Nutrient intake between self-reported dietary groups was comparable, although the vegan dietary group consumed significantly less dietary cholesterol ($p<0.001$), saturated fat ($p=0.012$), DPA ($p=0.002$) and DHA ($p=0.007$) compared to the LOV group after Bonferroni correction. No differences were observed between self-reported athlete status, diet duration, education level, formal nutrition training and nutrient profiles.

Table 4.3. Nutrient intake of self-reported plant-based athletes (n=133)

	Median	IQR (25-75%)
Energy (kJ)	8897.3	6982.1 - 11820.4
Protein (g)	72.0	56.4 - 97.2
Total Fat (g)	82.7	55.5 - 115.5
Carbohydrate (g)	235.5	182.4 - 316.8
Fibre, total dietary (g)	46.2	34.0 - 64.6
Sugars, total (g)	95.3	58.1 - 129.0
Moisture (g)	3120.3	2004.5 - 3957.7
Alcohol (g)	0	0 - 0.8
Caffeine (mg)	96.0	22.6 - 219.9
Fatty acids, total saturated (g)	18.4	11.6 - 29.1
Fatty acids, total polyunsaturated (g)	20.0	11.8 - 30.4
Fatty acids, total monounsaturated (g)	32.5	21.2 - 50.3
22:6 n-3, Docosahexaenoic acid [DHA] (mg)	1.7	0 - 16.0
22:5 n-3, Docosapentaenoic acid [DPA] (mg)	4.4	0.7 - 18.1
20:5 n-3, Eicosapentaenoic acid [EPA] (mg)	1.7	0.0 - 10.6
18:3, Alpha-Linolenic acid (g)	2.4	1.2 - 3.5
18:2, Linoleic acid (g)	16.7	10.6 - 27.1
Cholesterol (mg)	36.9	5.9 - 115.5
Vitamin E, alpha-tocopherol (mg)	14.3	10.7 - 20.7
Total Vitamin E (mg)	18.3	11.9 - 28.8
Beta-carotene (mcg)	4743.9	1636.0 - 10986.3
Preformed vitamin A (retinol) (mcg)	128.1	42.6 - 253.4
Vitamin A, RAE (mcg_RAE)	1099.9	534.5 - 2461.6
Vitamin B-12 (mcg)	1.2	0.4 - 2.2
Folate, DFE (mcg_DFE)	701.8	500.7 - 939.6
Folate, natural (mcg)	478.9	286.8 - 712.1
Folic acid (mcg)	112.0	6.6 - 176.7
Folate, total (mcg)	611.6	432.9 - 844.4
Vitamin B-6 (mg)	1.6	1.0 - 2.2
Niacin (mg)	19.2	13.5 - 24.0
Riboflavin (mg)	1.5	1.0 - 2.0
Thiamin (mg)	1.5	1.1 - 2.0
Vitamin C (mg)	154.4	88.4 - 271.4
Selenium (mcg)	63.3	43.3 - 94.9
Zinc (mg)	10.5	8.0 - 15.0
Sodium (mg)	2360.8	1601.3 - 3615.4
Potassium (mg)	3959.9	2737.2 - 5320.6
Phosphorus (mg)	1375.6	1086.6 - 1969.4
Magnesium (mg)	536.7	398.7 - 720.5
Iron (mg)	16.8	11.6 - 22.7
Calcium (mg)	822.8	578.8 - 1193.1

Table 4.4. Diet quality summary as assessed using the Alternate Healthy Eating Index and Dietary Photochemical Index Score (n=133).

	Mean	Std deviation
AHEI-2010 Score	70.3	10.76
Males	72.1	9.94
Females	69.5	11.05
Phytochemical Index Score (%)	65.7	22.17
Males	71.2	20.71
Females	63.4	22.46

The mean AHEI-2010 score was 70.3 (10.76 S.D) and the mean DPI score was 65.7 (22.17 S.D; **Table** 4.4). Both men and women consumed the suggested amount of wholegrains (men 233.6grams, women 124.6grams), nuts and legumes (men 3.7, women 1.8 serves/day), sugar-sweetened beverages and fruit juice (men 0.0, women 0.0 servings/day), red and processed meats (men 0.0, women 0.0 serves/day) and trans-fat (men 0.2, female 0.3 % of total daily energy intake) as per the AHEI-2010 **Table** 4.5**,** Neither men or women achieved the recommended number of servings of vegetables (≥5 serves; men 3.3, women 2.89 serves), fruit (≥4 recommended; men 2.7 serves, women 1.3) or mg/day of LC n3 PUFA's (250mg/day recommended; men 8.6mg/day, women 13.1).

Table 4.5. Diet quality as measured by the Alternate Healthy Eating Index-2010 (n=133).

Component	Criteria for minimum score (0)	Criteria for maximum score (10)	Component score Male (n=40)	AHEI-2010 Score Male (n=40)	Component score Female (n=93)	AHEI-2010 Score Female (n=93)
Vegetables (servings/day)	0	≥5	3.3 (2.3-5.5)	6.7 (4.7-10.0)	2.89 (1.9-4.6)	5.6 (3.9-8.9)
Fruit (servings/day)	0	≥4	2.7 (0.5-5.3)	6.6 (1.2-10.0)	1.3 (0.2-2.9)	3.2 (0.5-7.0)
Whole grains (g/day)						
Women	0	75			124.6 (48.1-266.3)	10.0 (6.4-10.0)
Men	0	90	233.6 (73.8-446.5)	10 (8.2-10.0)		
Sugar-sweetened beverages and fruit juice (servings/day)	≥1	0	0.0 (0-0.5)	9.9 (4.8-10.0)	0.0 (0.0-0.2)	9.9 (7.9-10.0)
Nuts and legumes (servings/day)	0	≥1	3.7 (2.0-5.6)	10.0 (10.0-10.0)	1.8 (0.8-3.5)	10.0 (8.3-10.0)
Red/processed meat servings/day	≥1.5	0	0.0 (0.0-0.0)	10.0 (10.0-10.0)	0.0 (0.0-0.0)	10.0 (10.0-10.0)
Trans fat (% of energy)	≥4	≤0.5	0.2 (0.1-0.4)	10 (10.0-10.0)	0.3 (0.1-0.5)	10.0 (10.0-10.0)
Long-chain omega-3 fatty acids (DHA/EPA; mg/day)	0	250	8.6 (0.1-67.1)	0.3 (0.0-2.7)	13.1 (2.4-57.8)	0.5 (0.1-2.2)
PUFA (% of energy)	≤2	≥10	8.1 (5.5-9.6)	7.6 (4.4-9.5)	7.8 (5.3-10.9)	7.2 (4.2-10.0)
Sodium (mg/day)*	Highest decile	Lowest decile	2584.0 (1365.6-4794.4)	4.6 (1.2-8.2)	2360.8 (1710.5-3429.3)	5.1 (2.8-7.2)
Alcohol(drinks/day)						
Women	≥2.5	0.5-1.5			0.0 (0.0-0.7)	2.5 (2.5-2.5)
Men	≥3.5	0.5-2.0	0.0 (0.0-0.6)	2.5 (2.5-2.5)		
Total	0	110		71.9 (64.0-79.0)		71.8 (61.8-78.7)

Values reported as median and IQR (25-75%). PUFU; Polyunsaturated Fatty Acids

Weak correlations were observed between the DPI and the AHEI-2010 for age (r=0.083 & 0.027), BMI (r=-0.055, -0.072), total MET minutes (r= -0.048, -0.096) or total sitting minutes respectively (r=-0.089, 0.088; **Table** 4.6). Similarly, no relationship was observed between any of the respondents' demographic groups and either of the diet quality tools (**Table** 4.7 and **Table** 4.8). When associations were explored between dietary motives and diet quality a significantly improved diet was observed for those who followed a vegetarian-based diet with the aspiration to improve performance in both the AHEI (p=0.01) and the DPI (p=0.03; **Table** 4.9). No other significant associations were observed between diet quality and dietary motivations.

Table 4.6. Correlations between, respondent demographics, dietary motives, dietary behaviours and diet quality measured by the Phytochemical Index and AHEI-2010 (n=133).

	Phytochemical Index Score	**AHEI-2010 Score**
Age	0.083	0.027
BMI	-0.055	-0.072
Total MET-Mins Total	-0.048	-0.096
Total Sitting Minutes	-0.089	0.088

Table 4.7. Relationship between AHEI-2010 dietary scores and demographic groups and athlete status.

	Mean	Std Deviation	P Value
Sex			
Males	72.07	9.94	0.21^
Females	69.50	11.05	
Education			
Secondary School	71.90	7.61	0.21
Diploma or Equivalent	66.50	9.60	
University Undergraduate	71.43	11.68	
University Honours/Masters	68.24	10.83	
Doctorate	73.39	9.34	
Age Group			
20-29	69.24	12.23	0.50
30-39	70.98	10.20	
40-49	71.78	8.76	
50-59	69.75	14.20	
60-69	58.47	5.18	
Diet duration			
0-6 Months	68.55	10.97	0.67
6-12 Months	74.06	12.06	
1-2 Years	68.70	15.30	
2-5 Years	70.57	8.86	
5-10 Years	68.06	8.53	
10-20 Years	72.25	12.54	
20+ Years	72.41	8.61	
Self-reported athlete status			
Competitive	71.68	8.97	0.52
Recreational	69.41	11.71	
Professional	71.77	13.55	
Formal Nutrition Training^			
No	69.70	10.98	0.18^
Yes	73.05	9.28	

P value between group analysis performed using one-way ANOVA. ^ p value calculated using independent samples t test.

Table 4.8. Relationship between dietary phytochemical index scores and demographic groups and athlete status.

	Mean	Std Deviation	P Value
Sex			
Males	71.28	20.71	0.06^
Females	63.41	22.46	
Education			
Secondary School	69.97	19.41	0.28
Diploma or Equivalent	73.98	26.93	
University Undergraduate	67.99	21.00	
University Honours/Masters	59.83	22.45	
Doctorate	66.78	20.82	
Age Group			
20-29	61.12	20.69	0.32
30-39	68.88	20.65	
40-49	65.42	23.66	
50-59	69.40	31.22	
60-69	76.01	33.93	
Diet duration			
0-6 Months	65.30	26.81	0.95
6-12 Months	67.84	23.85	
1-2 Years	66.16	22.64	
2-5 Years	68.04	20.78	
5-10 Years	62.48	24.55	
10-20 Years	67.52	24.72	
20+ Years	61.87	19.56	
Self-reported athlete status			
Competitive	64.01	23.55	0.63
Recreational	66.53	21.86	
Professional	76.17	17.33	
Formal Nutrition Training			
No	65.21	22.96	0.54^
Yes	68.39	17.98	

P value between group analysis performed using one-way ANOVA. ^ p value calculated using independent samples t test.

Table 4.9. Dietary phytochemical Index and AHEI-2010 dietary scores between motives for following a vegetarian-based dietary pattern.

AHEI-2010				
Reason for following diet	**n**	**Mean**	**Std Deviation**	**P Value**
Health				
No	32	70.30	8.40	0.98
Yes	99	70.25	11.45	
Weight loss				
No	110	70.66	10.83	0.34
Yes	21	68.19	10.39	
Animal rights				
No	20	70.60	11.24	0.88
Yes	111	70.20	10.72	
Environment				
No	35	70.16	9.74	0.95
Yes	96	70.30	11.15	
Improve performance				
No	94	68.70	11.10	0.01*
Yes	37	74.24	8.76	
Always followed diet				
No	127	70.10	10.87	0.32
Yes	4	75.54	4.01	
Religion/Spirituality				
No	121	70.41	10.92	0.60
Yes	10	68.52	8.79	
Dietary Photochemical Index				
Reason for following diet	**n**	**Mean**	**Std Deviation**	**P Value**
Health				
No	32	60.41	17.73	0.12
Yes	100	67.44	23.24	
Weight loss				
No	111	66.86	20.28	0.18
Yes	21	59.78	30.25	
Animal rights				
No	20	67.99	22.84	0.62
Yes	112	65.34	22.13	
Environment				
No	35	66.10	21.02	0.91
Yes	97	65.61	22.68	
Improve performance				
No	94	63.06	21.38	0.03*
Yes	38	72.35	22.99	
Always followed diet				
No	128	65.73	21.98	0.99
Yes	4	65.83	31.94	
Religion/Spirituality				
No	122	66.14	22.47	0.47
Yes	10	60.86	18.46	

AHEI-2010, Alternate healthy eating index 2010; Std Deviation, Standard deviation.
P-value between groups performed using independent samples t test

4.5 Discussion

The diet quality in these self-reported athletes following vegetarian-based diets tended to be of a high quality with the nutrient profile appearing generally appropriate to support athletic pursuits. The nutrient profile in this population group was collectively high, LC n-3 PUFA and vitamin B-12 intake was low and highlights the need for athletes following vegetarian-based dietary patterns to carefully consider their intake of these nutrients. Whilst the focus of this thesis is on vegetarian-based dietary patterns overall, it is useful from both an exercise performance perspective, and nutritional perspective (where some nutrients have previously been highlighted as more difficult to obtain whilst following a vegetarian-based diet [44]) to discuss some of nutrients individually in the context of the dietary pattern.

4.5.1 Nutrient Status

4.5.1.1 *Calcium*

Calcium's role for athletes extends beyond bone health with its importance recognised for both cardiac and skeletal contractions, nerve impulses, blood pressure regulation, nutrient transportation, and intracellular signaling among others [310]. Exercise does not inherently increase calcium requirements, although there may be an increased loss during perspiration [311] whilst a decrease in urinary calcium excretion may be observed with exercise [312]. For the athlete following a plant-based diet it has been suggested that a similar intake of calcium be consumed to their omnivorous counterparts [313]. The self-reported athletes in this study had a median of 822.8mg/day which was greater than the mean calcium intake of Australians (805mg/day) in 2011/12 [314]. The intake in this group was modestly lower than other studies examining the nutrient status in trained endurance athletes following a vegan

diet (median 973mg/day; Chapter 6) and in recreational runners (903mg/day)[315] yet substantially higher than mixed athletes from Brazil (482.3mg/day)[316]. Whilst the bioavailability of calcium in some plant-based foods can be lowered due to the oxalate composition [44], endurance athletes eating a vegetarian-based diet can readily meet their calcium requirements via consumption of low-oxalate vegetables such as bok choy and kale and an array of other vegetables, tofu, pulses, nuts, seeds and fortified plant-milks [43]. These foods should be a focus for the athlete following a vegetarian-based dietary pattern to ensure their calcium requirements are met.

4.5.1.2 *Zinc*

Athletes have been shown to have significantly lower serum zinc concentrations than non-athletes despite an increased dietary consumption suggestive of increased requirements for those who are physically active [317]. It has also been suggested that individuals following vegetarian-based dietary patterns consume 150% of recommended dietary intakes due to the inhibitory effects of phytates on zinc absorption [318]. Although, the suggested increased zinc intake in vegetarian-based populations has been determined using data from single meal studies and fails to consider the body's long-term compensatory mechanisms of those following vegetarian-based diets [319]. Despite lower dietary zinc intake being observed in those following vegetarian-based diets [320], serum and plasma zinc status is comparable to non-vegetarian based groups [321-324] or modestly reduced [320]. It appears there is a physiological adaption following adoption of a long-term vegetarian-based diet likely due to reduced endogenous zinc excretion coupled with improved absorption efficiency [319]. In this study, the median zinc intake was 11.5mg for males and 10.1mg/day for females which aligns with the EAR of 12mgday for males and 6.5mg for females [318]. These results are comparable to other studies involving athletes following vegetarian-based diets ranging from

7.7 to 12.87mg/day [315, 316]. Although, similarly to non-athlete populations where omnivorous dietary patterns are associated with increased dietary zinc intake compared to vegetarian-based, the dietary intake of zinc in the athletes following vegan diets in this study as well as others, appears to be lowered compared to athletes following omnivorous diets [315, 317]. Zinc is implicated in an assortment of pathways related to exercise adaptions [317], and evidence suggests that poor zinc status can negatively affect physical activity [325]. Therefore, athletes following vegetarian-based diets should ensure their dietary intake of zinc is high by including an abundance of whole foods rich in zinc such as nuts, seeds, tofu, legumes and wholegrains.

4.5.1.3 *Iron*

Iron intake in this vegetarian-based population group (16.8mg/day) was modestly less compared to recreational runners (18.4mg/day) [147] and self-reported male trained endurance athletes (19.85mg/day; Chapter 6) following vegetarian-based diets. Whilst iron intake appears generally sufficient in this population and in other studies examining athletes following plant-based diets, the bioavailability of iron in vegetarian-based groups is of particular interest. The absorption of non-heme iron has been suggested to be considerably less than heme-iron (up to 70%). Although, decreases in fecal ferritin excretion have been observed in those following vegetarian-based dietary patterns suggestive of a physiologic adaptation to increase the efficiency of iron absorption in these populations [326]. As athletes are at greater risk for iron deficiency due to exercise induced iron losses [327], and serum ferritin concentrations have been observed to be reduced in vegetarian-based populations [328], athletes following vegetarian-based dietary patterns should give careful consideration to their dietary iron intake.

Whilst supplementing iron may increase ones haemoglobin mass leading to improved oxygen

delivery [329], unless iron deficient anaemia is present, supplementation may not be advisable. Iron supplementation increases serum ferritin, which has been associated with increased risk of non-communicable diseases [330, 331] and iron induced oxidative stress [332]. Athletes following vegetarian-based diets should instead plan their intake to include an abundance of nutrient dense, whole foods, rich in iron such as dark leafy greens, tofu, soybeans, chickpeas, lentils and most nuts and seeds. Additionally, as phytic acids can inhibit non-haem iron absorption [333], increasing the bioavailability of iron from plant-foods can be considered. Food preparation procedures such as soaking and sprouting can considerably increase the bioavailability, whilst consumption of vitamin C and other organic acids such as citric, malic and lactic acids with meals can increase absorption of non-haem iron by 6-fold in those who have low iron stores [334].

4.5.1.4 *Iodine*

Unfortunately, iodine intake was not available on the ASA24-AU tool however has been included in this discussion due to its importance in vegetarian-based dietary patterns. Iodine is not typically associated as a mineral implicated in athletic pursuits, although is important for athletes following vegetarian-based dietary patterns to consider for several reasons. Low iodine status and deficiency prevalence has been shown to be higher in vegetarian, and particularly in vegan population groups due to the omission of animal based foods in their diet and soil depletion contributing to low concentrations found in plant-based foods [43, 335]. Further, considerable amounts of iodine can be lost via sweat during physical activity [336]. Indeed, iodine deficiency is not solely confined to vegetarian-based populations with the World Health Organization declaring iodine deficiency a major public health issue for populations throughout the world in 2004 prompting salt iodisation in many countries [337]. Seaweeds can bolster iodine intake for those consuming vegan-based dietary patterns, with

some vegan cohorts exhibiting 'excessive' iodine concentrations due to their high seaweed consumption [335]. Adding excessive iodised salt to meals is not recommended due to its association with non-communicable disease [338], however, if salt is used (which often is in endurance athletes to replace sodium lost in sweat [339]), iodised salt should be. Supplementation of iodine might be considered for athletes choosing to follow a vegetarian-based diet who choose to avoid additional sodium intake and seldomly consume seaweed products.

4.5.1.5 *LC n-3 PUFA*

Groups who follow a vegetarian-based dietary patterns have been shown to have lowered levels of dietary LC n-3 PUFA intakes compared with groups who eat fish [340]. LC n-3 PUFA are anti-inflammatory [341], have an inverse relationship with cardiovascular risk [342, 343], can modulate heart rate and heart rate variability [344, 345] and may play a role in ameliorating exercise induced physiological strain [346]. Despite the lowered LC n-3 PUFA intake typically observed in those following vegan dietary pattern, an inverse association between those consuming a vegan dietary pattern and heart disease risk [30, 347, 348] and risk of CVD [348, 349] exist. Nonetheless, as the results of this study indicate the median LC n-3 PUFA intake (3.4mg/day) was drastically under the adequate intake for DHA+EPA (Men 160mg/day – Women 90mg/day [350]) those engaging in physical activity and following a vegetarian-based diet should carefully consider their LC n-3 PUFA intake. Future research could consider examining LC n-3 PUFA's intake dietary intake and omega-3 index in these population groups and determine if there's any apparent exercise related physiology relationship.

4.5.1.6 *Vitamin B12*

Both athletes and non-athletes following vegetarian-based and in particular vegan diets must regularly consume foods fortified with vitamin B12 or specifically supplement the vitamin as few plant sources contain traces of vitamin B12 and long-term adherence to a vegetarian-based diet can result in a deficiency with considerable repercussions [44, 291]. The present study demonstrated insufficient intake in this self-reported vegetarian-based population whereby the median intake was 1.2mcg/day whilst the EAR is 2.0mcg/day [351]. From Study 1a it could be seen that a substantial proportion of self-reported athletes following a vegetarian-based diet supplement vitamin B12 (58.1%), but this should be increased to 100% to ensure adequate intake. Athletes and non-athletes alike following a vegetarian-based dietary pattern should supplement vitamin B12.

4.5.1.7 *Vitamin D*

Vitamin D was similarly not able to be obtained via the ASA24-AU dietary collection tool. Nonetheless a brief discussion is warranted due to the vitamin previously being highlighted as a nutrient of concern for individuals following vegetarian-based diets. Vitamin D is important for a range of functions in the human body and has a particular interest for the athlete due to its involvement in muscle metabolism [352, 353]. The vitamin is predominately obtained via UV light from sunlight [354], but is also obtained to a lesser extent from the diet. Foods containing Vitamin D are scarce, with only a few naturally occurring foods containing the vitamin and even fewer plant-based foods plentiful in vitamin D [355]. Vitamin D insufficiency is a concern for athletes following both vegetarian-based and non-vegetarian diets with almost 50% of the world's population with insufficient vitamin D [356] concentrations. Athletes can achieve vitamin D sufficiency by exposing their skin to a few minutes of sunlight daily [357] or supplementing (1,000-2,000 IU/day). If supplementation is preferred, athletes following vegan based diets should look for vitamin D-3 which is derived

from lichen rather than lanolin (an oily compound obtained from sheep's wool) [358].

4.5.1.8 *Protein*

Protein recommendations for individuals differs in accordance with training level and exercise type. For example, the recommended dietary intake for Australians engaging in light to moderate activity is 0.84g/kg for males and 0.75g/kg for females. Protein requirements are increased for the athletes engaging in regular physical activity with ~1.4g/kg suggested for endurance athletes and up to 2g/kg for strength athletes [50, 359]. More recently it has been suggested that timing of protein might be more important for muscle protein synthesis than focusing on a single target to reach daily with 0.3g/kg suggested to be consumed after key workouts and consumed every 3-4 hours [359]. It was previously thought that some plant foods were 'incomplete' lacking certain essential amino acids thereby foods had to be carefully considered and consumed to ensure a complete amino acid profile was achieved each day by those following vegetarian based dietary patterns [44]. This is a myth however with all plant foods containing the 9 essential amino acids [360] and providing caloric intake is adequate, vegetarian-based diets meet or exceed protein recommendations in non-athlete populations [44, 361]. There is little evidence suggesting athletes following vegetarian-based diets require an increased protein intake [358] compared to those following omnivorous diets. Protein requirements for athletes are easily achieved on vegetarian-based diets providing an array of whole foods from a variety of food groups are included to ensure all nine essential amino acids are consumed in sufficient quantities [44]. It is difficult to interpret protein intake in the present study as there were a range of sporting types, intensities and abilities in this cohort, although with a median intake of 1.18grams of protein consumed per day it is likely the majority of respondents consumed sufficient protein for moderate activity. The values were consistent with other studies including other athletes following vegan diets with

1.11g/day/kg body weight in healthy physically active women [362], 1.61g/day of body weight in ultrarunners [59] (vegan and LOV combined) and 1.25g/kg body weight in recreational runners [315] and athletes following LOV diets with 1.34g/kg [315].

4.5.1.9 *Carbohydrates*

As described in Chapter 1, optimising carbohydrate consumption for endurance performance has been recommended to assist in prolonging submaximal activity, assist in reducing muscle damage, and help improve recovery [50, 52, 53]. Specifically, it is suggested for those engaging in ~ 1 hour of moderate activity individuals should consume 5-7grams of carbohydrates per kilogram per day. The median carbohydrate intake in the current study was under this recommendation at 3.6g/kg/day. Carbohydrate intake in vegetarian-based populations is generally high and exceeds that of non-vegetarian dietary patterns [38, 147, 148]. Several explanations may account for the lower-than-expected carbohydrate intake observed in the present study. Firstly, the respondents engaged in an array of sports, many of which were not endurance-based. Carbohydrate intake may have purposely been lowered in some respondents engaging in particular sports where high carbohydrate intake is not as important for performance compared to others (i.e., weightlifting vs running). Second, dietary data was self-reported. Under-reporting in self-reported dietary collection is a common limitation [363] and may have undervalued true carbohydrate intake. Finally, dietary data was collected using the ASA24-AU, a tool created for an Australian context. Some foods consumed by respondents outside of Australia may have been unavailable for selection thereby impacting carbohydrate values.

4.5.2 Diet Quality

4.5.2.1 *AHEI-2010*

The median AHEI-2010 score in this study appeared to be high at 71.9/110 when compared to other cohorts assessed using the AHEI-2010. For instance, in the Nurses' Health Study (n = 73 228), the median AHEI-2010 was 62.1 in the highest quintile [303], whilst the median AHEI-2010 in a nationally representative sample of 29,124 adults aged 20 to 85 years in 2010 in the United States of America was 46.8 [305]. Other studies have shown that adoption of a vegan-based diet can significantly improve AHEI-2010 scores while other therapeutic diets, such as the American Diabetes Diet may not [364]. A recent systematic literature review assessing diet quality in plant-based diets found that in the majority of studies (9/12) those adhering to a plant-based diet had a higher overall diet quality compared with non-vegetarians. In general the plant-based diets tended to more closely align with recommendations for total fruit, whole grains, plant protein, and sodium but were less likely to adhere to recommendations for refined grains and total "protein foods" [33].

4.5.2.2 *Dietary Phytochemical Index*

The DPI has been associated with a lower prevalence of cardiometabolic disease [308], improved waist circumference, lowered systolic and diastolic blood pressure [365], improved total blood lipid concentrations, lower total and HDL-cholesterol concentrations [366] and reduced insulin resistance and prediabetes [367, 368]. A higher DPI has also been inversely associated with C-reactive protein, a common marker of inflammation [369]. This may have relevance for athletes as reducing inflammation during exercise may have ergogenic effects [370]. It is difficult to contrast the DPI scores obtained in this survey to the aforementioned studies due to disparate demographics. For instance, the respondents in this study were self-reported athletes who volunteered for the trial likely exaggerating the DPI scores compared to non-athletes disinterested in research and their health outcomes. Nonetheless, to our

knowledge this is the first study to investigate DPI in both athletes and those following a self-reported vegetarian-based eating pattern which provides a foundation for future exploration using this tool whilst demonstrating the high intake of phytochemicals in this cohort.

4.5.3 Relationship between dietary motive and diet quality

Radnitz et al [162] explored the difference in health behaviours in individuals following a vegan diet for either ethical or health reasons and reported those choosing a vegan diet for health reasons would make other healthier choices compared to those following for ethical reasons. In the present study, those who identified as following a vegetarian-based diet to 'improve exercise performance' scored significantly higher on both the DPI and AHEI-2010 indicating healthier diet patterns overall when compared to athletes following the diet due to other motives. It may therefore be important for health professionals to consider individuals motivations for vegetarian based adherence as these may impact upon nutritional and health outcomes.

4.6 Limitations and strengths

Nutrient data has been obtained using the ASA24-AU which has been specifically created for an Australian context and may become less appropriate for use in countries outside of Australia. Sodium scoring within the AHEI-2010 is more suited towards the use in large cohorts to find associations with risk factors rather than small studies such as the present one. Additionally, iodine and vitamin D, two nutrients that have been noted as being potentially harder to obtain for those follow vegetarian-based dietary pattern were not available within the ASA24-AU. Finally, due to the nature of this study, participants were self-selected and self-reported their dietary intake and as such, is not a representative sample. On the other

hand, to the candidates knowledge this study has provided the largest pool of vegetarian-based athletes assessing diet quality and nutrient status.

4.7 Conclusion

This study has shown that athletes choosing to follow a vegetarian-based diet to improve performance may have improved diet quality compared to those who follow a vegetarian-based diet for other reasons. Whilst more research is required, the current study has also demonstrated that a vegetarian-based dietary pattern can provide a high diet quality for athletes with a sufficient nutrient status for physical activity. Some nutrients were insufficient however and require special attention when planning a suitable vegetarian-based diet for athletes which include vitamin B12 and LC n3 PUFA's. A focus on the consumption of whole foods for athletes following vegetarian-based rather than refined and processed foods should optimise their diet quality and nutrient profiles for performance.

Although this study showed evidence that a vegetarian-based dietary pattern may provide a robust nutrient profile and high diet quality, the cohort was comprised solely of self-reported vegetarian-based participants with no comparator dietary group. To understand the impact of a vegetarian-based dietary pattern on physiological markers such as inflammatory and immune responses, a comparison of vegetarian and non-vegetarian based diets is required, as outlined in chapter 5.

5 Vegetarian-based dietary patterns and their relation with inflammatory and immune biomarkers: A systematic review and meta-analysis | Study 2

A substantial portion of this chapter has been published.

Craddock JC, Neale EP, Peoples GE, Probst YC, (2020). Vegetarian-Based Dietary Patterns and their Relation with Inflammatory and Immune Biomarkers: A Systematic Review and Meta-Analysis. Advances in Nutrition 1:10(3):433– 451. DOI: 10.1093/advances/nmy103

5.1 Introduction

As described in chapter 1, nutritional epidemiology has seen a shift away from single nutrient analyses to a complementary approach in the form of dietary pattern analyses [1]. Evaluating dietary patterns may provide a more holistic and clinically relevant approach to assessing diet-disease relation as nutrients are not eaten in isolation and synergistic effects of multiple components can have a concerted effect [3]. Vegetarian-based dietary patterns are typically higher in fruits, vegetables, wholegrains, nuts, seeds and legumes, all of which are naturally higher in phytochemicals and some vitamins compared to non-vegetarian dietary patterns [44, 138]. Consumption of these dietary patterns are protective against many conditions [15, 371-373].

An array of mechanisms are likely responsible for the protective effects observed in vegetarian-based dietary patterns, including improved inflammatory and immune responses, as discussed in Chapter 1. These systems can be modulated by various dietary pattern and food components, demonstrating that plant-based foods can provide favorable outcomes [39, 110, 374, 375]. For instance, without an adequate supply of nutrients the immune system may be weakened [75]. In addition, the impact of 'non-nutritive' components of food on immune function has been acknowledged [376-378]. For example, polyphenolic compounds are shown to improve lymphocyte responsiveness and natural killer cell function [80] while carotenoids can have an immune modulating effect [379]. The influence of diet on inflammation has also been examined with clear associations found [110, 240]. Consumption of dietary patterns with substantive nutrients obtained from plant sources compared to animal sources have been shown to attenuate markers of chronic inflammation such as CRP, Interlukin-6 (IL-6) and fibrinogen [39, 110, 240, 380] although limited research examining these relationships has been performed. When considering the implications of these findings,

it should be noted that we do not consume these components in isolation [3]. As such, exploration of the impact of consuming a whole dietary pattern that is high in these components as often seen in vegetarian-based seems indicated.

As observed in study 1b, the diet quality in self-reported athletes following vegetarian-based diets tended to be of a high quality with a robust nutrient profile including several antioxidant compounds such as vitamin C, vitamin E and beta-carotene within the dietary pattern. However, intake of LC n-3 PUFA was low in this population group. Given the limited research focusing specifically on athletes, exploring the evidence on the collective effects of vegetarian and non-vegetarian based dietary patterns on inflammation and immune markers in adults more broadly is required to determine if differences in diet can influence important physiological markers. Understanding this relationship in the broader population is necessary to inform the development of later studies to test the effect of vegetarian-based eating patterns in athletes. It was therefore the aim of this study to systematically review the evidence to determine if vegetarian-based eating patterns in humans are associated with, or able to modulate, inflammation or immune biomarkers compared to those following non-vegetarian dietary patterns. A meta-analysis was performed to explore the effect of vegetarian-based eating patterns on common inflammation and/or immune biomarkers compared to non-vegetarian dietary patterns.

5.2 Methods

5.2.1 Study protocol

The systematic review was reported according to the Preferred Reporting of Systematic Reviews and Meta-analyses (PRISMA, **Appendix B**) statement [381] and was registered with

the International Prospective Register of Systematic Reviews (PROSPERO, CRD42016039043; 12 May 2016). A systematic search of the Pubmed, MEDLINE and Cochrane Central Register of Controlled Trials scientific databases (all years to December 2017) was conducted to answer the research question. Scientific database searches were conducted by one reviewer (JC). The search strategy used the following key words, with Medical Subject Heading terms used where available: (“Immunoglobulin*” OR “IgE” OR “IgD” OR “IgM” OR “IgA” OR “IgG” OR “Platelet*” OR “Basophil*” OR “Eosinophil*” OR "t lymphocyte subsets" OR “t cell*” OR "b lymphocyte subsets OR “B cell*” OR “Monocyte*” OR “Neutrophil*” OR “Lymphocyte*” OR “Leukocyte*” OR "white blood cell*" OR “NK” OR "natural killer t cell*" OR "natural killer cell*" OR "immunity" OR “immune” OR “tumor necrosis factor” OR "tumour necrosis factor" OR “TNF” OR “interleukin” OR “IL-6 “ OR “fibrinogen” OR OR “CRP” OR "c reactive protein" OR “C-Reactive Protein” OR “inflammat*”) AND ("plant based" OR "plant-based" OR “vegan*” OR “*vegetarian” OR “vegetarian*”). An example of the search strategy in its entirety is shown in **Appendix C**. This review considered any dietary pattern that included animal meats (including fish) to be non-vegetarian-based while dietary patterns excluding all animal meats were considered to be vegetarian-based.

5.2.2 Inclusion criteria

Studies were included if they examined the relationship (observational studies) or effect (intervention studies) of vegetarian-based dietary patterns compared to a non-vegetarian-based control dietary pattern on an outcome of interest (CRP, interleukins (all), TNF (all), fibrinogen, natural killer cells, white blood cell counts (Leukocytes, Lymphocytes, Neutrophils, Monocytes, Eosinophils, Basophils, Thrombocytes), Immune globulins (IgG, IgA, IgE, IgD and IgM), and were conducted in human populations of all ages.

Observational studies were defined a priori to include any studies in which there was no direct intervention and could include; cross-sectional, case-control, prospective cohort and retrospective cohort studies. Studies had to additionally; involve participants who had adhered to a vegetarian-based diet (vegetarian group only) for at least one year. This timeframe was chosen to represent a habitual dietary pattern.

Intervention studies were also defined a priori to include any studies where a vegetarian-based diet was used as an intervention with a control group and could include randomised controlled trials, non-randomised controlled trials and pre-post studies. Intervention studies had to additionally; study the vegetarian-based diet for a period exceeding four weeks. This timeframe was selected as changes in some serum inflammatory markers such as IL-6 and CRP can take several weeks to become physiologically apparent [236, 237, 382].

5.2.3 Exclusion criteria

Observational and intervention studies were excluded if they; (1) were not published in the English language, (2) were conference abstracts, editorials, book series, erratum and conference proceedings, (3) did not complete between group analyses or provide raw data to allow this to be calculated, (4) were animal or cellular models, (5) were analysing consumption of single foods or food groups rather than dietary patterns (e.g. exploring legume intake rather than vegetarian diets), (6) used drugs which could alter biomarker outcomes i.e. metformin (CRP) [383, 384], (7) were assessing antibodies to food antigens rather than disease or general blood immunoglobulins, (7) included any type of animal meat (including fish) in the vegetarian-based groups or (8) examined a single diet component/supplement only (e.g. cheese vs vegan cheese alternate). Intervention studies were additionally omitted if they; (1) used lifestyle interventions in conjunction with diet

intervention i.e. exercise and/or stress management, (2) used intervention diets containing any type of meat and/or not reporting to control/discouraged meat intake.

Duplicate articles were initially removed using EndNote software (version X7, 2013 Thomson Reuters; Philadelphia, Pennsylvania) with any remaining duplicates removed manually within Endnote. Articles were firstly screened based on title and abstract. Full text articles were obtained if the abstract was unavailable, or if it was unclear if the article met the inclusion criteria. Screening was performed by reviewer JC with articles of concern discussed amongst the research team (YP, EN, GP) until consensus was reached. Where results from the same study were reported in multiple articles, the most recent article was included to avoid duplication of results. Reference lists of included articles were manually searched to identify any additional relevant articles.

5.2.4 Data extraction

Data extraction was performed by reviewer JC in consultation with the research team and included information related to: author, date, study design, level of evidence, study population (including age, gender, country and co-morbidities), sample size, length of vegetarianism (observational studies), type of vegetarianism, details of intervention and control groups (intervention studies), outcomes investigated and significant differences in biomarkers. Study authors were contacted for additional details if the required data was not available in the published article.

5.2.5 Statistical analysis

Meta-analysis were performed when more than three studies reported on a biomarker, median/mean with standard deviation could be obtained or calculated from raw data and the units of measurement could be made uniform. Meta-analyses were conducted separately for

observational and intervention study results. Review Manager software (Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014) was used to estimate the pooled effect of inflammation and immune markers between vegetarian and non-vegetarian diets. Random effect meta-analyses were conducted to determine weighted mean differences by assigning a weight to each study on the basis of an individual study's inverse variance [385]. 95% confidence intervals were used for each outcome. If a study involved more than one intervention group meeting the inclusion criteria, data for all intervention groups were combined as recommended by the Cochrane Handbook [386]. For the intervention analysis, cross-over studies were initially analysed as parallel studies using a paired analysis, the most conservative approach to managing cross-over studies [386]. Paired analyses of cross-over studies using correlation coefficients of 0.25, 0.5 and 0.75 were then conducted as sensitivity analyses to determine if this influenced the results [386]. The I^2 statistic was used to evaluate heterogeneity with a score 50 - 90% likely indicating substantial heterogeneity and a score of 75% - 100% considerable heterogeneity [386]. Where ≥ 10 studies reported on a biomarker outcome, funnel plots were generated and Egger's test applied to assess studies for small study effects [230] using StatsDirect statistical software (Version 3.1, England: StatsDirect Ltd, England, 2013) [387]. Where median and ranges were reported, the Hozo et al formula was used to calculate standard deviation and/or mean (when the population was < 25 persons) [388]. When interquartile range (IQR) was given, IQR /1.35 was used to calculate standard deviation [386]. Where insufficient information was described in the published article and raw data was provided by authors, statistical analyses were performed using SPSS software (v21, SPSS Inc, Chicago, Il 2012). Shapiro-Wilk tests were applied to raw data to determine if biomarker outcomes were normally distributed. One-way ANOVA (parametric) or Kruskal-Wallis (non-parametric) tests determined if differences existed between dietary patterns for inclusion in

the summary table. *P* values < 0.05 were considered to be statistically significant. Sensitivity analyses were performed by excluding each study individually to investigate the influence on overall estimates [386]. Additionally, sensitivity analyses were conducted by excluding studies where participants suffered from a chronic condition. When sufficient data were available for type of vegetarianism (LOV or vegan dietary patterns) (≥ 3 studies) sub-group analyses were performed.

5.2.6 Risk of bias

Study quality for the non-randomised studies was assessed independently using a modified version of the Newcastle-Ottawa Scale (NOS) by two reviewers (JC, EN). Where discrepancies occurred, a third reviewer (YP) was consulted until a consensus was reached. The NOS for each study was based on the primary outcome of the present study (CRP) if available. For intervention studies, risk of bias was assessed using the Cochrane Collaboration's tool [386]. To determine the quality of the body of evidence, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) method was applied to both observational and intervention studies [35].

5.3 Results

The literature search identified 2040 articles. After the exclusion criteria were applied, 39 studies (30 observational articles [86, 88, 89, 92, 94, 136, 389-412] describing 29 studies (two separate articles were identified reporting on same study participants, with different outcome marker/s) and 8 intervention studies) were included in the review. A further two studies were identified via manual searching of reference lists, resulting in a total of ten intervention studies [95, 413-421]. **Figure 5.1** displays the process of study selection including identification, screening, eligibility, and inclusion.

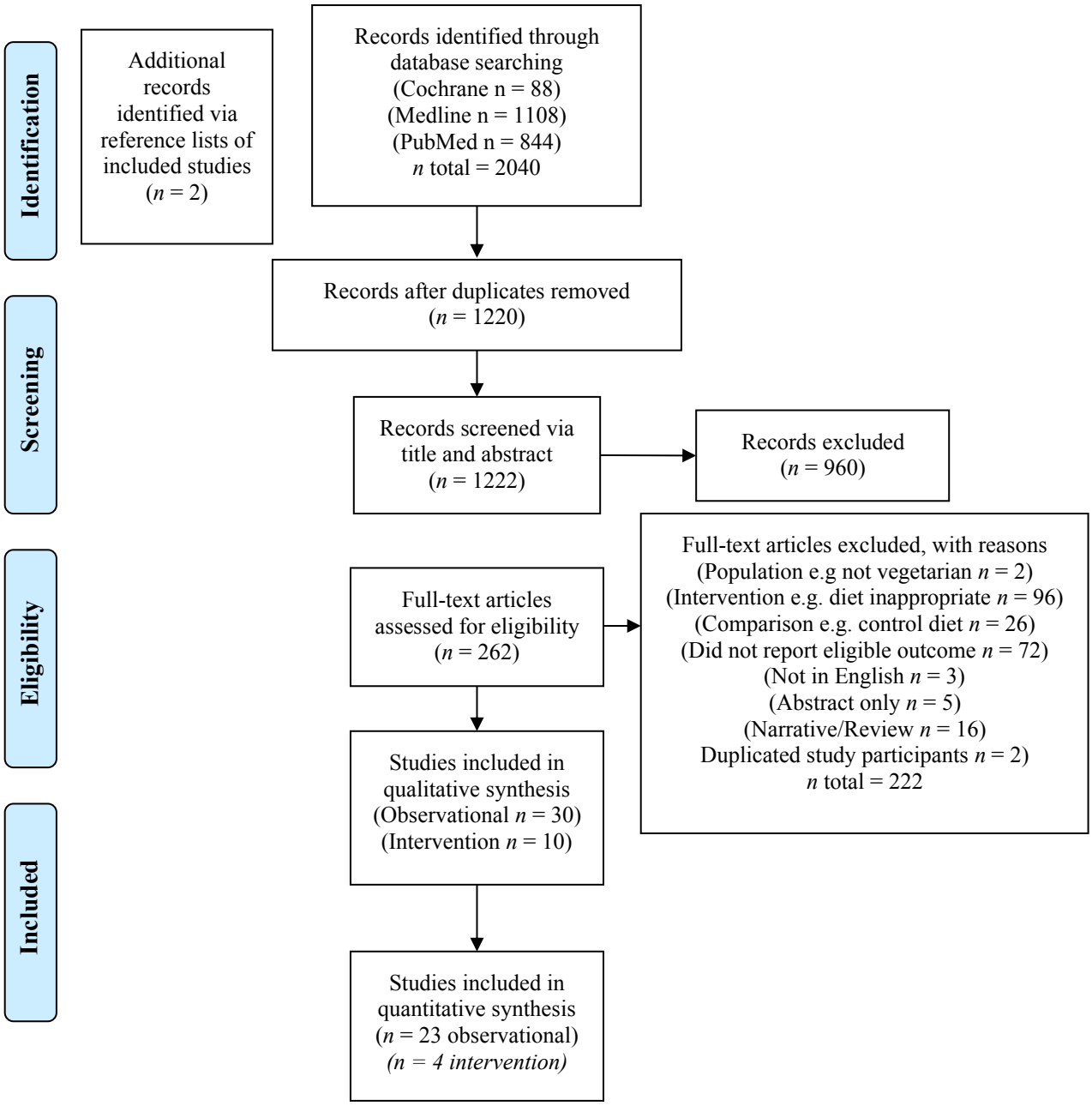


Figure 5.1. Flowchart of study selection.

5.3.1 Observational Studies

The included studies were cross-sectional or matched cohort studies (**Table** 5.1). Types of vegetarianism included LOV (8), Lacto-vegetarian (2), vegan (5) and combinations of these with comparison groups typically consuming mixed omnivorous non-vegetarian diets. Participants in two of the included studies had chronic conditions with one receiving dialysis therapy [403, 411] while participants in the other study had cardiovascular disease and/or diabetes mellitus [406]. One study [406] reported on participants whose age ranged between 2-18 years old whilst the remainder reported on adults aged 18 years or older (**Table** 5.1). Studies were conducted in a range of continents including: Asia [86, 92, 136, 391-393, 403, 406, 409-411, 422], Africa [394] North America [88, 395, 396, 399, 404], South America [89, 397, 401, 423] and Europe [94, 398, 400, 402, 407, 408, 424, 425]. CRP concentrations were significantly lower in 9/19 studies in the vegetarian-based groups, with no difference in 10/19 studies [136, 391-393, 395-397, 399-401, 403, 404, 407-409, 411, 422-424]. Leukocyte counts were significantly lower in 6/11 studies in the vegetarian-based groups with no difference in 5/11 studies [86, 88, 89, 92, 94, 391, 392, 399, 407, 410, 411] (**Table** 5.2). Four studies reported on lymphocyte counts with vegetarian-groups displaying significantly lower counts in two of the studies [86, 92, 94, 399]. Only two studies reported on NK cell cytotoxic activity as a function of applied immune-competence and found improved function in the vegetarian-based group [94] or no difference between groups [399]. One study reported lower neutrophil counts in vegetarian-based groups [89], while the other three studies found no difference between groups (54, 62, 69). Fibrinogen was observed to be lower in vegetarian-based groups in 2/3 studies [394, 401, 410]. **Table** 5.2 shows the number of included studies identified for each biomarker and summarises the number of studies reporting significant and non-significant differences in outcomes between vegetarian and non-vegetarian groups. Study

quality ranged from 2-6 out of a possible seven using the modified NOS tool (**Appendix D).**

Table 5.1. Characteristics of observational studies examining the association of participants following vegetarian-based or non-vegetarian dietary patterns and common biomarkers of inflammation and immune function.

Study	Design/Level of Evidence	Population/n size/Gender	Co-morbidities	Country	Age (years; mean or range)	Years on vegetarian diet	Biomarker/s investigated	Study Quality /7	Matched (NS different at baseline)	Difference in biomarker (significance = P<0.05)
Acosta-Navarro et al. [423]	Cross sectional	V - 44	Nil	Brazil	V[3] = 45.5	> 4	hs-CRP	5	age	● hs-CRP – NS[4]
Navarro et al. [2] (48)		NV - 44			NV = 46.8		Leukocytes		gender	● Leukocytes significantly ↓ in V group
		M					Neutrophils		smoking status	● Neutrophils significantly ↓ in V group
									hx of disease	
Ambroszkiewicz et al. [424]	Cross sectional	LOV - 43	Nil	Poland	4.5–9.0	> 4.5	CRP	4	age	● CRP - NS
		NV - 46							BMI	
		M & F								
Chen et al. [392]	Cross sectional	V - 99	Nil	Taiwan	V = 51.24	> 1	Leukocytes	4	age	● CRP - significantly ↑ in NV group
		NV - 99			NV = 49.38		hs-CRP		BMI	
		M & F								
Chen et al. [391]	Cross sectional	LOV - 173	Nil	Taiwan	LOV = 54.00	> 1	hs-CRP	5	BMI	● hs-CRP - NS
		NV - 190			NV = 49.94		Leukocytes			● Leukocytes - NS
		F					Thrombocytes			● Thrombocytes - NS
Chuang et al. [393]	Matched cohort/Cross sectional	V - 686	Nil	Taipei & Taiwan	V = 45.2	Long-term	CRP	5	age	● CRP significantly ↑ in NV group
		NV - 3423			O = 45.1				location	
		M & F							sex	
Dong and Scott. [88]	Cross sectional	Ve - 13	Nil	United States of America	M = 57 | F = 40	> 1	Leukocytes	1	Nil	● Significance not reported
		LV - 28			M = 45 | F = 42					
		LOV - 15			M= 43 | F = 35					

		NV - 4			M = 31 | F = 55					
Famodu et al. [394]	Cross sectional	M & F Ve - 8	Nil	Nigeria	Ve = 47.1	Long-term	Fibrinogen	3	age BMI	● Fibrinogen significantly ↑ in NV group compared to LOV & Ve group.
		LOV -28			LOV = 49.0					● Fibrinogen significantly ↑ in LOV group compared to Ve group
		NV - 40			NV = 48.7					
		M & F								
Fontana et al. [395]	Matched Cohort/Cross sectional	Ve - 21	Nil	United States of America	Ve = 53.1	> 2	hs-CRP	2	age	● CRP significantly ↑ in NV group.
		NV - 21			NV = 53.1					
		M & F								
Fontana et al. [396]	Matched Cohort/Cross sectional	Ve - 18	Nil	United States of America	54.2	1½ - 10 (range)	hs-CRP	2	age	● hs-CRP significantly ↑ in NV group.
		NV - 18							sex	
		M & F							SES	
Franco-de-Moraes [397]	Cross sectional	Ve - 66	Nil	Brazil	Ve = 49.6	> 1	TNF-α	3	age	● TNF-α - NS
		LOV - 102			LOV = 49.6		CRP		sex	● hs-CRP significantly ↑ in NV group
		NV - 100			NV = 49.1		IL-10			● IL-10 - NS
Gorczyca et al. [398]	Cross sectional	V = 22	Nil	Poland	V = 4	> 1	IgA	3	age	● IgA - NS
		NV = 18			NV = 9 (range 2 - 18)		IgM		body weight	● IgM - NS
		M & F					IgG		height	● IgG - NS
Haddad et al. [399]	Cross sectional	Ve - 25	Nil	United States of America	Ve = 36.0	> 1	Leukocytes	3	age blood lipid concentrations.	● Leukocytes significantly ↓ in Ve group
		NV - 20			O = 33.5		Lymphocytes		physical activity level	● Lymphocytes significantly ↓ in Ve group

		M & F					Neutrophils			● Neutrophils - NS
							Monocytes			● Monocytes - NS
							Eosinophils			● Eosinophils - NS
							Basophils			● Basophils - NS
							Thrombocytes			● Thrombocytes significantly ↓ in Ve group
							IgA			● IgA - NS
							IgG			● IgG - NS
							IgM			● IgM - NS
							CRP			● CRP - NS
							NK cell cytotoxic activity			● NK cell cytotoxic activity - NS
Krajcovicova-Kudlackova, & Blazicek [400]	Cross sectional	LOV - 133	Nil	Slovakia	LOV = 46.2	>1	hs-CRP	2	age	● hs-CRP significantly ↑ in NV group.
		NV - 137			NV = 47.2					
		M & F								
Malter et al. [94]	Cross sectional	V = 22	Nil	Germany	V = 28-50	> 1	Thrombocytes	2	age	● Thrombocytes - NS
		O = 22					Leucocytes		gender	● Leucocytes - NS
		M					Lymphocytes			● Lymphocytes - NS
							Monocytes			● Monocytes - NS
							Basophilic granulocytes			● Basophilic granulocytes - NS
							Eosinophilic granulocytes			● Eosinophilic granulocytes - NS
							NK cell cytotoxic activity			● NK cell activity of peripheral blood lymphocytes significantly ↑ in V group.
Mezzano et al. [401]	Cross sectional	V = 26	Nil	Chile	V = 39	> 1	Platelet count	3	age	● Thrombocytes significantly ↓ in NV group
		NV = 26					Fibrinogen		sex	● Fibrinogen significantly ↑ in NV group
		M & F					CRP		SES	● CRP - NS

Montalcini et al. [402]	Cross sectional	LOV = 26	Nil	Italy	LOV = 32.6	≥ 3	IL-2	5	age	● IL-2 - NS
		NV = 26			NV = 30.5		IL-4		BMI	● IL-4 - NS
		M & F					IL-6		gender	● IL-6 - NS
							IL-8			● IL-8 - NS
							IL-10			● IL-10 - NS
							TNFα			● TNFα - NS
							IL-1α			● IL-1α - NS
							IL-1β			● Interleukin-β significantly ↑ in LOV group
Ou et al. [403]	Case control/Cross sectional	V = 21	Patients on dialysis therapy for >6 months.	Taiwan	V = 56.27	≥ 1.5	hs-CRP	2	age	● hs-CRP - NS
		NV = 42			O = 56.29				sex	
		M & F								
Paalani et al. [404]	Cross sectional	V - 216	Nil	United States of America	68.8	> 1	CRP	4	Not reported	● CRP significantly ↑ in NV group
		NV - 289					IL-6			
		M & F					IL-10			
							TNF-α			
Pinto et al. [425]	Matched Cohort/Cross sectional	Ve - 23	Nil	United Kingdom	Ve = 49	> 2	Il-6	6	age	● IL-6 - NS
		NV - 24			NV = 54				sex	
		F							BMI	
Pongstaporn et al. [86]	Cross sectional	V = 179	Nil	Thailand	V = 18+	> 1	Leukocytes	2	Nil	● Leukocytes significantly ↓ in Ve group
		NV = 58					Thrombocytes			● Thrombocytes - NS
		M & F					Neutrophils			● Neutrophils - NS
							Lymphocytes			● Lymphocytes - NS

Refsum et al. [406]	Cross sectional	V = 78 NV = 126 M & F	100 CVD (42 of which DM) 104 without CVD (41 DM)	India	V = 27–55	Long-term	Thrombocytes	3	Nil	● Thrombocytes - NS
Sebekova et al. [407]	Cross sectional	LOV = 90 NV = 46 M & F	Nil	Slovakia	LOV = 37.7 O = 37.1	> 2	Hs-CRP Leukocytes	2	age gender BMI	● Hs-CRP - NS ● Leukocytes - NS
Sebekova et al. [408]	Cross sectional	Ve = 19 LOV = 19 NV = 9 M & F	Nil	Slovakia	Ve = 39.6 LOV = 36.1 NV = 30.5	Ve = 7.2 LOV = 8.2	CRP	2	age	● CRP - NS
Su et al. [409]	Cross sectional	LOV = 49 NV = 41 F	Nil	Taiwan	LOV = 58.6 ± 6.0 O = 57.2 ± 5.4	10.8	hs-CRP	3	age gender	● hs-CRP - NS
Suwannuruks et al. [410]	Cross sectional	LOV = 50 NV = 30 M & F	Nil	Thailand	LOV 18-50	>1	Fibrinogen Leukocytes Thrombocytes	1	Nil	● Fibrinogen - NS ● Leukocytes - NS ● Thrombocytes - NS
Szeto et al. [136]	Cross sectional	LOV = 30 NV = 30 M & F	Nil	Hong Kong	LOV = 44.2 NV = 44.0	5-55 (range)	hs-CRP	2	age sex	● CRP significantly ↑ in NV group
Tungtrongchitr et al. [92]	Cross-sectional	LV = 132 NV = 47 M & F	Nil	Thailand	LV M = 35.5 | F = 33 NV M = 32.5| F = 32 Median	>1	Leukocytes Neutrophils Lymphocytes	2	age sex SES	● Leukocytes - NS ● Neutrophils - NS ● Lymphocytes significantly ↓ in LV group

							Monocytes		ethnic origin	● Monocytes - NS
							Eosinophil			● Eosinophils significantly ↓ in female LV group compared to male LV and NV group.
							Basophil			● Eosinophils significantly ↑ in male LV group compared to female LV group and NV group
							Thrombocytes			● Basophils - NS
										● Thrombocytes - NS
Wu et al. [411]	Cross sectional	V = 19	Patients receiving dialysis therapy > 6 months	Taiwan	V = 63.3	Long before HD - Note mean length of HD = 5.9	hs-CRP	4	age	● hs-CRP significantly ↑ in NV group
		NV = 299			NV = 57.5		Leukocytes		sex	● Leukocytes significantly ↓ in V group
		M & F							mean HD length	
Yang et al. [422]	Matched cohort/Cross sectional	V = 171	Nil	China	V = 32.6	> 1	CRP	4	age	● CRP - NS
		NV = 12			NV = 34.2					
		M								

[1] CVD, Cardiovascular Disease; DM, Diabetes Mellitus; HD, Haemodialysis; LOV, Lacto-ovo-vegetarian; LV, Lacto-vegetarian; NR, Not Reported; NS, Not Significant; NV, Non-vegetarian; SES, Socio-Economic-Status; Ve, Vegan. [2.] Two separate papers identified reporting on same study participants, with different outcome marker/s – Slight difference in Navaro et al's population; V – 43, NV – 41, age; V = 45.0, NV = 46.5 and study quality; = 4 between studies. [3.] V, Participants followed a combination of Ve and/or LV and/or LOV. [4.] NS, Not Significant = P > 0

Table 5.2. Included studies reporting on biomarkers and significant differences between participants following vegetarian-based or non-vegetarian dietary patterns in observational studies.

Biomarker	Studies Included	Differences between groups (significance = $P < 0.05$)
Lymphocytes [86, 92, 94, 399]	4	↓ in V group in 2/4 studies | NS 2/4 studies
Neutrophils [86, 89, 92, 399]	4	↓ in NV group in 1/4 studies | NS 3/4 studies
Basophils [92, 94, 399]	3	NS 3/3 studies
Monocytes [92, 94, 399]	3	NS 3/3 studies
Eosinophils[3] [92, 94, 399]	3	NS 3/3 studies
NK cell cytotoxic activity [94, 399]	2	↑ in V group in 1/2 studies | NS 1/2 studies
Leukocytes [86, 88, 89, 92, 94, 391, 392, 399, 407, 410, 411]	11	↓ in V group in 6/11 studies | NS 5/11 studies
Thrombocytes [86, 92, 94, 391, 399, 406, 410]	7	↓ in V group in 1/7 studies ↑ in V group in 1/7 studies | NS 5/7 studies
CRP [136, 391-393, 395-397, 399-401, 403, 404, 407-409, 411, 422-424]	19	CRP ↓ in veg group in 9/19 | NS 10/19 studies
TNF-α[4] [397, 402, 404]	3	NS
Fibrinogen [394, 401, 410]	3	↑ in NV group in 2/3 studies | NS 1/3 studies
Interleukins		
IL-10 [397, 402, 404]	3	NS
IL-6 [402, 404, 425]	3	NS
IL-2, IL-4, IL-8, IL-1α,IL-1β [402]	1	IL-1β ↑ in V group in 1/1 study
Immunoglobulins		
IgA, IgM, IgG [398, 399]	2	NS

[1] NV, Non-Vegetarian; V, Vegetarian-based.
[2] NS, not significant between groups ($P > 0.05$)
[3] Tungtrongchitr et al compared medians between groups and genders. Eosinophils were significantly ↓ in the NV group compared to the male LV group but significantly ↑ compared to the female LV group.
[4] Significance not reported in one study.

5.3.1.1 *Relation between vegetarian-based diets on inflammatory and immune biomarkers*

Twenty-six observational studies were included in the meta-analysis reporting on four outcomes: CRP, thrombocytes, leukocytes, and fibrinogen (**Table** 5.3). Consumption of a vegetarian-based dietary pattern was associated with significantly lower CRP ($P = 0.001$; **Figure 5.2**), fibrinogen ($P = 0.02$; **Figure 5.3**) and leukocyte ($P = 0.02$; **Figure 5.4**) levels compared to those following a mixed omnivorous non-vegetarian comparison diet. No significant difference was observed for thrombocytes between groups ($P = 0.16$; **Figure 5.5**). The quality of body of evidence for the observational studies was deemed to be 'very low' after a one level downgrade was applied for each outcome as per the GRADE guidelines [35] (**Appendix E**). Funnel plots were generated for CRP and leukocyte concentrations. Egger's test indicated no significant asymmetry (**Appendix F & G**).

Table 5.3. Meta-analysis summary of observational studies comparing CRP, thrombocytes, fibrinogen and leukocytes between vegetarian-based and non-vegetarian based dietary patterns.

Outcome	Number of analyses	Number of Vegetarian participants	Number of control participants	Effect Estimate (95% CI)	P value	Inconsistency (I^2)	GRADE Quality
CRP (mg/L)	18	1844	4736	-0.61 (-0.91, -0.32)	0.001	100%	Very Low
Thrombocytes (x10^9/L)	7	663	507	8.24 (-3.35, 19.82)	0.16	35%	Very Low
Fibrinogen (g/L)	3	112	96	-0.22 (-0.41, -0.04)	0.02	17%	Very Low
Leukocytes (10^3/µL)	11	944	970	-0.62 (-1.13, -0.10)	0.02	96%	Very Low

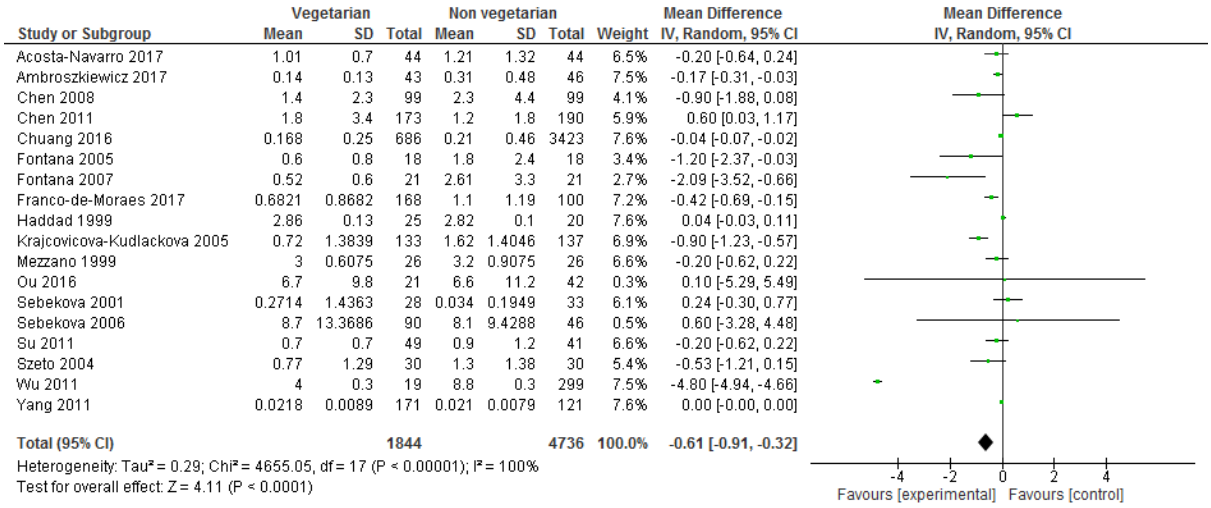


Figure 5.2. Difference in CRP values between participants following vegetarian-based dietary patterns and non-vegetarian dietary patterns (cross-sectional studies). Diamond indicates weighted mean difference with 95% confidence intervals.

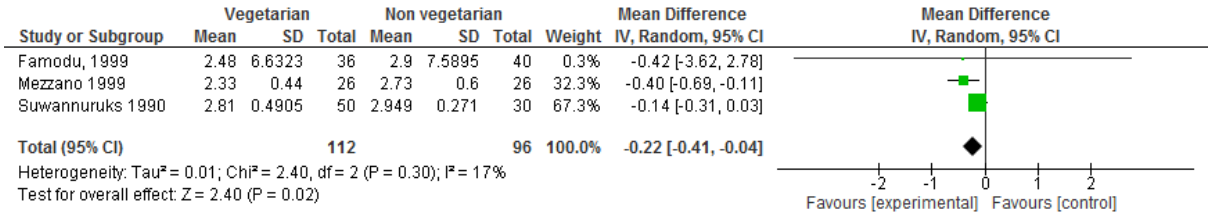


Figure 5.3. Difference in fibrinogen values between participants following vegetarian-based dietary patterns and non-vegetarian dietary patterns (cross-sectional studies). Diamond indicates weighted mean difference with 95% confidence intervals.

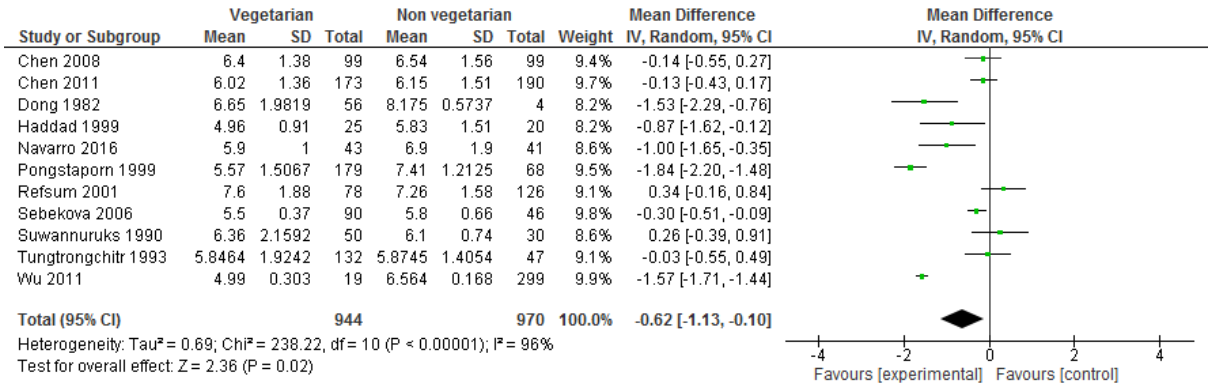


Figure 5.4. Difference in leukocyte values between participants following vegetarian-based dietary patterns and non-vegetarian dietary patterns (cross-sectional studies). Diamond indicates weighted mean difference with 95% confidence intervals.

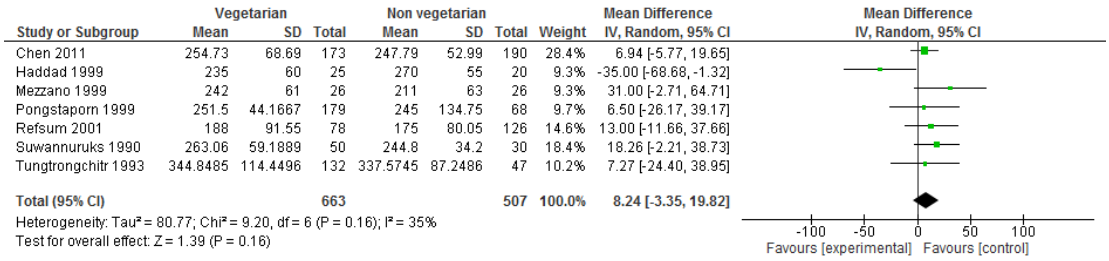


Figure 5.5. Difference in thrombocyte values between participants following vegetarian-based dietary patterns and non-vegetarian dietary patterns (cross-sectional studies). Diamond indicates weighted mean difference with 95% confidence intervals

5.3.1.2 *Sensitivity analysis and subgroup analysis*

When sensitivity analyses were applied, the pooled effect on CRP remained significant. The pooled effect on leukocytes became non-significant when Pongstaporn et al [86] was omitted (P = 0.08; **Appendix H**). Conversely, thrombocytes were significantly higher in the vegetarian group with the omission of Haddad et al [399] (P = 0.01) (**Appendix I**). Lower leukocyte and CRP levels in vegetarian-based populations continued to be found when sensitivity analyses were applied excluding studies with participants receiving haemodialysis treatment, cardiovascular disease (CVD) and/or T2DM (0.01) (**Appendix J).**

Due to considerable heterogeneity observed (I^2 = 100%) for CRP concentrations between dietary groups, meta-analyses were performed on specific dietary groups in an attempt to identify the source of heterogeneity. No significant sub-group differences were observed between vegan, LOV groups and non-vegetarian groups for CRP. Neither sub-group analysis accounted for the high heterogeneity (I^2 for both vegan and LOV groups 87%; **Appendix K & L.**

5.3.2 Intervention Studies

Ten intervention studies were identified exploring the effect of vegetarian-based eating patterns on common markers of inflammation and/or immune function [95, 413, 414, 416-418, 421, 426-428]. They included seven parallel and three crossover intervention study designs. Of the included studies, seven were randomized [413, 414, 416-418, 421, 426], and the remaining three were unable to be confirmed as being randomized or non-randomized [95, 427, 428] as authors could not be contacted. Vegetarian-based intervention diets included LOV (*n*=3), LV (*n*=1) and vegan (*n*=6) with varying macronutrient percentages (**Table** 5.4). Control diets varied and included; a well-balanced mixed diet from the five food groups [413], a conventional T2DM diet recommended by the European Association for the Study of Diabetes [414], habitual mixed diets [416, 418, 426-428], and an American Heart Association diet (Fat total 30%, 7% saturated fat, < 300 mg of cholesterol, < 1500 mg of sodium daily) [417]. Intervention diet duration ranged from four to 56 weeks. Studies were from North America [417, 421] and Europe [95, 413, 414, 416, 418, 426-428]. The populations examined in the included studies were mixed. For instance, in four studies the participants had rheumatoid arthritis, one study population exhibited T2DM, one study participants were overweight or obese (class 1; as measured by BMI) and one study participants were children > 95% of BMI for age. Biomarkers investigated varied between studies (**Table** 5.4).

CRP levels were found to be significantly lower in the vegetarian-based groups compared to the non-vegetarian groups in 4/7 studies, with no significant difference in 3/7 intervention studies (**Table** 5.5). Lymphocytes, monocytes, Pan T cells (CD3+), T suppressor cells (CD8+), T helper cells (CD4+), NK cells, TNF-α, fibrinogen, IL-6 and IgA were reported by

only one intervention study, with no significant difference between vegetarian and non-vegetarian groups found. **Table** 5.5 shows a summary of the included intervention studies and corresponding biomarker outcomes with significant and non-significant differences between study groups. The quality of body of evidence for the intervention studies was rated as 'very low' according to GRADE (**Appendix N**) [35].

Table 5.4. Characteristics of intervention studies examining the effect of vegetarian dietary patterns and non-vegetarian dietary patterns on common biomarkers of inflammation and immune function [1]

Study/Year	Study design/Level of Evidence	Population/ Gender	Comorbidities	Country	Age in years (mean or range)	Duration of vegetarian diet (weeks)	Intervention Vegetarian dietary pattern	Control Non-Vegetarian	Biomarker/s investigated	Matched (baseline participant characteristics matched)	Difference in biomarker (significance = $p<0.05$)
Elkan et al. [413]	RCT (Level II)[2]	Ve = 30	Patients with RA (2 and 10 years duration)	Sweden	Ve = 49.9	52	Ve	Well-balanced mixed diet from 5 food groups	CRP	Age	hs-CRP significantly ↓ *within* LV group
		NV = 28			NV = 50.8		Gluten Free	(CHO 55% to 60%,10%-15% Pro, Fat < 30% with < 10% saturated).		Weight	
		M & F					(CHO 60%, Pro 10%, Fat 30%)			BMI	
										Disease duration	
										Concomitant treatment	
Hunt & Roughead [421]	RCT (Level II; crossover)	*n* = 21	Nil	U.S.A	33.2	8 (nil washout)	LOV	LOV with ~184g meat (3 parts beef and 1-part chicken)/d	CRP	N/A - Crossover	CRP – NS
		F					↑ amounts of legumes, wholegrains, breads/cereals fruits and vegetables Cessation of supplements				

Kahleova et al. [414]	RCT (Level II)	LV = 37	Patients with T2DM	Czech Republic	LV = 54.6	12	LV	Conventional T2DM diet as per DNSG of the EASD	hs-CRP	Significant differences between groups not reported at baseline	hs-CRP significantly ↓ in LV group
		NV = 37			NV = 57.7		Animal products were limited to one low-fat yogurt a day.	(50% CHO, 20% Pro, < 30% fat; ≤ 7% saturated fat, < 200 mg⁄day of cholesterol).	Fibrinogen		Fibrinogen – NS
		M & F					(60% CHO, 15% Pro & 25% fat)				
Kjeldsen-Kragh et al[3]. [416, 426]	RCT (Level II)	Ve = 27	Classic or definite RA	Norway	Ve = 53	56	Ve	Habitual mixed diet	CRP	Significant differences between groups not reported at baseline	hs-CRP significantly ↓ in Ve group
		NV = 26			NV = 56		Gluten free Ve for 3.5 months. Followed by LOV for 9.5 months		Thrombocytes		Thrombocytes significantly ↓ in Ve group
		M & F							Leukocytes		leukocytes significantly ↓ in Ve group
									TNFα		TNFα - NS
									IgM		IgM significantly ↓ in Ve group
									IgA		IgA - NS
									IgG		IgG significantly ↓ Ve group (after 1 month

Macknin et al. [417]	RCT (Level II)	Ve = 14	Children BMI > 95th % for age/sex, cholesterol >169mg/dl	U.S.A	Children Ve = 15.0 O = 15.0	4	Ve	American Heart Association diet	hs-CRP	No significant difference in biomarkers at baseline	hs-CRP significantly ↓ in children on Ve
		NV = 14			Adults Ve = 46.5 O = 46.0		avoidance of added fat and limited intake of nuts and avocado.	(Fat total 30% , 7% saturated fat, < 300 mg of cholesterol, < 1500 mg of sodium daily)	IL-6		IL-6 – NS
		Overweight children with 1 x accompanying parent									
		M & F									
Nenonen et al. [418]	RCT (Level II)	Ve = 22	Chronic and active RA	Finland	Ve = 49.1	12	Ve	Habitual mixed diet	CRP	Height	CRP – NS
		NV = 21	CRP >10 mg/l		NV = 55.6		Rich in lactobacilli			Weight	
		M & F								BMI	
										Duration of RA	
										Seropositivity	
										Medication	
Richter et al. [95]	Non-randomized crossover design (Level III-2)[4]	*n* = 8	Nil	Denmark	21-28	12 (2 x 6 (crossover; 4 weeks washout)	LOV	High amounts of animal protein (CHO 57%, Pro 14%, Lipids 29%)	Monocytes conc	N/A - Crossover	Monocytes - NS
		well-trained male athletes					High in vegetable protein sources		Monocytes (CD14+)		Monocytes (CD14+) - NS

		M					(CHO 57%, Pro 14%, Lipids 29%)		NK cells (CD16+)		NK cells (CD16+) - NS
									Pan T cells (CD3+)		Pan T cells (CD3+) - NS
									T suppressor cells (CD8+)		T suppressor cells (CD8+) - NS
									T Helper cells (CD4+)		T Helper cells (CD4+) – NS
Sköldstam et al. [428]	RCT (Level II)	LOV = 15	Classical RA	Sweden	LOV = 35-56	12	LOV	Habitual mixed diet	Leukocytes	Not reported	Leukocytes - NS
		NV = 10			NV = 43-66		Nil alcohol, tobacco, coffee/ tea. Limited salt, sugar, white flour and grain products.		T lymphocytes		T lymphocytes - NS
		M & F							B lymphocytes		B lymphocytes - NS
									IgG		IgG - NS
									IgA		IgA - NS
									IgM		IgM significantly ↑ *within* LOV group
Sköldstam [427]	Non-randomized crossover design (Level III-2)	*n* = 20	Classical or definite RA	Sweden	35-68	16	Ve	Habitual mixed diet	CRP	N/A - Crossover	CRP – NS
		NR									

[1] CHO, Carbohydrates; F, Female; LOV, Lacto-ovo-vegetarian; LV, Lacto-vegetarian; M, Male; NR, Not Reported; NS, Not Significant ($P > 0.05$); NV, Non-vegetarian; Pro, Protein; RA, Rheumatoid Arthritis; T2DM, Type 2 Diabetes Mellitus; Ve, Vegan.
[2] RCT (Level II), Randomized Controlled Trial.
[3] Same study/participants - Different outcomes investigated.
[4] (Level III-2), A comparative study with concurrent controls: Non-randomized, experimental trial.

Table 5.5. Included studies reporting on biomarkers and significant differences between vegetarian and non-vegetarian based dietary patterns in intervention studies [1]

Biomarker	Studies Included	Differences between groups (significance = P <0.05)
Lymphocytes [428]	1	NS
Monocytes [95]	1	NS
Monocytes (CD14+) [95]	1	NS
Pan T cells (CD3+) [95]	1	NS
T Suppressor cells (CD8+) [95]	1	NS
T Helper cells (CD4+) [95]	1	NS
NK cells [95]	1	NS
Leukocytes [426, 428]	2	↓ in V group in 1/2 studies | NS 1/2 studies
Thrombocytes[426]	1	↓ in V group in 1/1 studies
CRP [413, 414, 417, 418, 421, 426, 427]	7	↓ in V group in 4/7 | NS 3/7 studies
TNF-α [416]	1	NS
Fibrinogen [414]	1	NS
Interleukins		
IL-6 [417]	1	NS
Immunoglobulins		
IgM [416, 428]	2	↓ in V group in 1/2 studies | ↑ *within* V group in 1/2 studies
IgA [416, 428]	2	NS
IgG [416, 428]	2	↓ in V group in 1/2 studies | NS 1/2 studies

[1] NS, not significant (P > 0.05); V, Vegetarian-based.

5.3.3 Pooled effects and subgroup analysis of vegetarian-based diets on inflammatory and immune biomarkers

Of the ten studies identified, only four studies were eligible for a meta-analysis examining vegetarian-based dietary patterns and their effect on CRP (vegetarian [*n* = 116], non-vegetarian [*n* = 114]). Due to the small population pool, varied population demographics (patients with rheumatoid arthritis, women, children with a BMI > 95th % for age/sex with cholesterol >169mg/dL and patients with T2DM), and varying intervention diets, the meta-analysis has been included as supplementary data to avoid potentially misleading conclusions common in nutritional meta-analyses [429] **(Appendix O)**. The Cochrane risk of bias assessment (**Appendix P**) and risk of bias graph (**Appendix Q**) are available as supplementary data. As a result of insufficient data and/or studies, it was not possible to perform meta-analyses for the other outcomes.

5.4 Discussion

To the candidates' knowledge, this review and meta-analysis is the first to explore both the association and effect of consuming a vegetarian-based dietary pattern on biomarkers of inflammation and immune status. The results of the analysis of observational studies suggest that individuals following vegetarian-based diets may have lower levels of CRP and fibrinogen, two prominent markers of inflammation, compared to their non-vegetarian based counterparts. Given CRP is implicated in the development of atherosclerosis [430] and is an independent risk predictor of cardiovascular events [431, 432], the results of this review may partly explain the lowered incidence of cardiovascular events observed in vegetarian populations [433, 434]. The lowered leukocyte and fibrinogen concentrations observed in vegetarian-based eating patterns appears to be favorable as elevated leukocyte and fibrinogen biomarkers have been associated with increased risk of all-cause mortality [96], T2DM [97],

metabolic syndrome [98] and coronary heart disease [435].

These results are in contrast to those of a previous meta-analysis, which found non-significant differences in CRP concentrations between vegetarian and non-vegetarian-based dietary patterns (Hedges' g = −0·15; 95% CI: 0·35, 0·05)[436]. There are several explanations for the inconsistency. Firstly, the present review excluded studies where statins were used by participants as they are known to reduce inflammation [383, 384] whereas the previous analysis included one study where statin use was significantly different between groups [437]. Secondly, the previous review [436] included studies that included small amounts of meats in the vegetarian group [438] or where the vegetarian dietary pattern was not adequately described [439], whereas these studies were excluded from our review. We also only included studies with a duration of vegetarianism of 1 year or longer, aligning with the suggestion that there may be a time interval between starting a vegetarian diet and reduction in CRP [436]. Finally, this review has included studies not available at the time of the previous review [397, 423, 424].

Despite ten intervention studies identified for inclusion in this review, many biomarkers of interest were not reported upon, or only explored in a single study thereby limiting conclusions regarding the effect of vegetarian-based dietary patterns on these outcomes. CRP was explored in seven studies however, with significantly lowered concentrations following consumption of a vegetarian-based diet observed in 4/7 studies, which aligns with the results of the observational meta-analysis presented here. The limited body of evidence identified in the intervention studies highlights the need for further RCTs to confirm the results of the observational meta-analysis. Similarly, no studies in the present review were identified focusing on athlete populations highlighting a gap in the literature. This warrants exploration,

particularly given the importance of the inflammatory responses during and post exercise outlined in Chapter 1.

An array of nutrients and 'non-nutritive' components of the vegetarian diet may be responsible for the trend for lowered inflammation biomarkers following consumption of a vegetarian-based dietary pattern [440]. Consumption of flavonoids such as quercetin, kaempferol, malvidin, peonidin, daidzein, and genistein have been inversely associated with serum CRP even after adjustment for covariates including vitamin C, vitamin E, carotenes, and fruit and vegetable consumption [441]. The antioxidant properties of flavonoids have been hypothesized to prevent LDL oxidation - an early inflammatory event in the development of atherosclerosis [442]. Similarly, carotenoids are potent antioxidants embedded within the lipid bi-layer functioning to appease free radicals and have been inversely associated with markers of inflammation [443, 444]. Both flavonoids and carotenoids are typically found in higher concentrations in those following vegetarian-based dietary patterns [445] and may contribute to the observed attenuation of inflammation in vegetarian-based groups. Phytochemicals, which tend to be more plentiful in vegetarian-based eating patterns [445], may act as antioxidant, antibacterial, antifungal, anti-inflammatory, antiallergic, hypotensive, chemo-preventive agents [375, 446], and may modulate inflammatory and immune function [375, 378]. Quantifying phytochemical intakes between vegetarian and non-vegetarian groups may be a target for future research.
Type and quantity of dietary fat intake may also influence low-grade inflammation concentrations. Several studies have linked dietary saturated fatty acids with increased serum hs-CRP and fibrinogen levels [118, 447]. Saturated fatty acid intake is typically higher in non-vegetarian based dietary patterns due to the increased consumption of animal based products [445] and may contribute to the increased concentration of serum CRP and

fibrinogen observed in non-vegetarian based populations. These patterns align with those observed in Study 1b, where saturated fat intake was below levels reported in the literature [445, 448]. Vegetarian-based populations typically consume a greater proportion of their dietary fat in the form of unsaturated fatty acids compared to non-vegetarians [449] which are inversely associated with inflammation [450]. It is important to note that overweight and obesity are associated with elevated inflammation markers including TNF-α and IL-6 [451]. Vegetarian-based populations typically exhibit lower BMI's than non-vegetarian populations [452] which may in part account for the reduced CRP, fibrinogen and total leukocyte concentrations in the vegetarian-based compared to the non-vegetarian based populations observed in this review.

Due to the limited number of studies, quantitative analysis was not possible for many biomarkers in both observational and intervention studies including interleukins (all), TNF-α, NK cell activity, lymphocytes, neutrophils, monocytes, eosinophils, basophils, IgG, IgA, IgD, IgE and IgM. Future research should investigating potential differences in these biomarkers between dietary groups. Interestingly, of the 2/4 studies which reported lowered total lymphocyte concentrations in vegetarian-based groups, both lymphocyte counts were within normal reference ranges (Haddad et al [399], $3.04 \pm 0.83 \times 10^9$/L, normal reference range $1.170 – 4.698 \times 10^9$/L,[99] and Tungtrongchitr [92] et al; 30% and 33% WBC (medians), normal reference range 18–54% [99]). If lymphocyte counts are reduced in vegetarian-based populations, yet NK cell cytotoxic activity is improved, the overall effect on immune function may be favorable. Further exploration into lymphocyte concentrations and NK cell activity in vegetarian-based populations is required.

While our review was comprehensive and systematic in nature, some limitations must be

noted. Our analysis was limited by the small number of studies assessing the effect of vegetarian-based dietary patterns on fibrinogen (*n*=3) and thrombocytes (*n*=7) in observational studies and CRP (*n*=5) in intervention studies. Furthermore, cross-sectional studies provide a high risk of bias and lower levels of study quality (compared to RCTs) [386]. However, inclusion of cross-sectional studies was warranted in this review to provide an estimation of vegetarian-based eating patterns and their relationship with a wide range of outcomes across a large population sample. In the case of this review, many studies used unit reporting methods which could not be converted to a common unit preventing their use in the meta-analysis, had limited sample sizes and often failed to control for risk factors which may have influenced inflammatory markers (e.g. BMI, physical activity and smoking status) which may have increased the risk of bias in these studies. Additionally, many of the observational studies lacked detail on the types and quality of diet in both vegetarian and non-vegetarian groups which presents challenges when interpreting the results of these studies. As mentioned, there was substantial variation between population groups and a small population sample pool in the intervention study quantitative analysis limiting the generalisability of the results. Furthermore, it was unclear if three of the intervention studies were randomized or not.

There are also several strengths of this review. This meta-analysis is the first, to the authors knowledge, to systematically and quantitatively assess the relation between vegetarian-based dietary patterns and biomarkers of inflammation and immune status in both observational and intervention studies. Previous studies have investigated the effects of specific nutrients and foods on markers of low-grade inflammation however nutrients and foods are seldom eaten in isolation [39, 440]. A strength of this review is that dietary patterns were considered as a whole which considers the complex synergistic and/or antagonistic biochemical interactions,

enhancing the applicability to real-life eating patterns [1].

5.5 Conclusion

This study systematically assessed the evidence including observational and intervention studies comparing common biomarkers of inflammation and immune status in vegetarian-based and mixed non-vegetarian dietary patterns. Vegetarian-based dietary patterns appeared to be favorable in all quantitative syntheses; however, results should be interpreted with caution due to the limited number of studies and substantial variation between studies. Future research should focus on large scale intervention studies, exploring differences in immune function between vegetarian-based and non-vegetarian based groups. This is justified given the increased consumption of 'non-nutritive' immune modulating phytochemicals typically consumed in vegetarian-based dietary patterns. Furthermore, since it appears there are favorable inflammatory profiles in vegetarian-based populations it is plausible immune function may also be improved given the inherent link between the two physiological systems.

This study has demonstrated significant differences in several markers of inflammation between vegetarian and non-vegetarian dietary patterns when all individuals (athletes and non-athletes) were considered. Given the importance of inflammation and immune responses during exercise and the increasing interest in consuming vegetarian-based dietary patterns in athletes it is important to consider the impact of these dietary patterns in athletes, however this systematic review has demonstrated that there is a lack of literature in this area. Further, there may also be other physiological relevant markers of exercise physiology differing between athletes following vegetarian and non-vegetarian diets. There is therefore a need to compare a range of physiological markers in athletes following vegetarian and non-vegetarian

dietary patterns, which studies 3 and 4 will explore.

6 Dietary intake, nutrient status and whole and red blood cell fatty acid profiles in trained male endurance athletes following a vegan or non-vegan eating pattern | Study 3

A substantial portion of this chapter has been published.

Craddock JC, Neale EP, Peoples GE, Probst YC, (2021). A Cross-Sectional Comparison of the Whole Blood Fatty Acid Profile and Omega-3 Index of Male Vegan and Omnivorous Endurance Athletes. Journal of the American College of Nutrition. Advanced online print. DOI: 10.1080/07315724.2021.1886196.

6.1 Introduction

Despite the apparent high prevalence of endurance athletes following vegan diets [57-59], there are limited quantitative studies evaluating the influence of these dietary patterns on diet and nutrient quality. Even less research has focused on exercise physiology outcomes in individuals following these diets, although as noted in chapter 1 there is theoretical evidence that this dietary pattern may modulate certain aspects of an endurance athlete's physiology. For instance, vegan dietary patterns are associated with higher carbohydrate intakes in the general population [36, 38]. Further, those following vegan dietary patterns often align with whole grain consumption recommendations more closely which is reflective of carbohydrate quality [33]. It has, therefore, been hypothesised that adhering to a vegan dietary pattern may optimize the glycogen concentrations thereby providing an advantage to endurance performance by delaying the time to fatigue [107, 149]. On the other hand, as noted throughout this thesis, some nutrients linked to reduced inflammation may be harder to obtain as part of a vegan dietary pattern such as long chain omega-3 fatty acids (LC n-3 PUFA) [43]. This evidence from the literature was observed within this thesis, whereby participants of Study 1b reported lower LC n-3 PUFA intakes than recommendations.

The provision of LC n-3 PUFA from the diet are recognised to contribute to a meaningful amelioration of physiological strain due to their role in the cell membrane; as long as fatty acids such as eicosapentaenoic acid (EPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are consistently consumed [292]. The LC n-3 PUFA, DHA and EPA are found predominantly in fish oil and marine animals which are excluded within a vegan pattern of eating. As a result, populations consuming a vegan eating pattern also typically have lower plasma concentrations of DHA [453], EPA [454] and subsequently omega-3 index (O3I); calculated using the sum of EPA and DHA in erythrocyte membranes expressed as a

percentage of total fatty acids, compared to those who include fish in their eating pattern [340, 455]. Most evidently, DHA is incorporated into the membranes of cardiac [456] and skeletal muscle fibres, especially fast-oxidative glycolytic fibres (type IIA) of the muscle according to dose [294]. Evidence from animal and human studies, describe the specific uptake of DHA into the membranes of the sarcoplasmic reticulum [457] and mitochondria [458] which then underpins improved skeletal muscle contractile efficiency [295].

The range of the O3I varies dramatically with differing eating patterns [459] demonstrating the importance of including LC n-3 PUFA in the diet. In the case of endurance athletes, one in three endurance athletes have been classified in the 'high risk' category for cardiac risk using the O3I (<4%) [459]. While LC n-3 PUFA are known to reduce cardiac risk, populations who consume vegetarian and vegan eating patterns that exclude food sources of LC n-3 PUFA's have been, however, associated with reduced heart disease risk [30, 347, 348] and risk of CVD [348, 349]. The reduced cardiac risk has been attributed to the vegetarian and vegan dietary patterns' ability to modulate a range of CVD risk factors including improved weight management, lowering systemic inflammation and reducing blood lipids and blood pressure [31, 32, 34, 40, 349, 460].

The results from Study 1b suggested athletes following a vegetarian-based diet may have a high diet quality assessed by the AHEI-2010 and phytochemical index. The nutrient analysis was also indicative of the diet providing sufficient nutrients to support physical activity, although, intake of some nutrients in this vegetarian-based population were insufficient, especially LC n3 PUFA's. Study 2 demonstrated that there appears to be significant differences in several inflammatory related biomarkers between those following a vegetarian compared to non-vegetarian based diet in general population groups. As the survey was online from a self-reported population, with only athletes following vegetarian-based diets,

and the systematic review was focused on the broader population (including non-athletes), a focused study under controlled conditions was required to substantiate the findings from these studies and further explore dietary behaviours between a group of endurance athletes following both a vegan and non-vegan dietary pattern. Endurance athletes were selected as it was hypothesised that the potential mechanisms for dietary differences to translate into disparate physiology would be most pronounced in this group as outlined in Chapter 1. It was, therefore, the aim of this study to explore dietary intake and nutrient status as well as whole and red blood cell fatty acid profiles in trained endurance athletes who follow a vegan or an omnivorous eating pattern.

6.2 Materials and Methods

6.2.1 Study protocol

This pilot trial explored the dietary fat intakes, whole blood fatty acids and O3I in trained endurance athletes following a vegan dietary pattern compared to matched (age, exercise volume, and aerobic capacity) athletes who follow an omnivorous eating pattern. Each participant attended the exercise physiology laboratory to collect a blood sample and complete the maximal aerobic capacity assessment on a treadmill. During the laboratory visit an Accrediting Practising Dietitian provided instructions about how to complete a 7-day food record.

6.2.2 Participants

Participants were recruited via Illawarra region vegan-based and non-vegan based athlete orientated Facebook groups and were requested to volunteer during a period of engaged training that represented the volume and intensity used to maintain normal performance (i.e., not the off season or pre-season). Participants were eligible for the study if they were: aged

between 18 and 55 years, engaged in a minimum of four hours of training per week, followed a vegan-based dietary pattern for at least 6 months (vegan group only) and were excluded if they were: smokers, suffering from, or had a history of, cardiac, hepatic, pulmonary, renal, neurological, haematological, psychiatric or gastrointestinal illness. Supplementation was permitted and outlined in participants food diaries. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) [461]. Ethics approval was granted by the University of Wollongong Human Research Ethics Committee (2018/258). All participants provided written informed consent to participate in the trial. **Appendix R** outlines the participant flowchart and communication between the research team.

6.2.3 Anthropometry

Upon arrival at the laboratory, the participant's body mass was measured in replicate in light clothing using mechanical scales (SECA mechanical column scale). Participant's height was measured with a stadiometer (SECA217 Stable Stadiometer, SECA, United Kingdom).

6.2.4 Dietary and Exercise Training Assessments

Each participant was required to record a 7-day consecutive detailed description of both their dietary intake and their exercise training. These records were regarded as usual practice for a sustained endurance training block and did not include either off-season or a taper period.

6.2.4.1 *Dietary collection and assessment*

The Australian 'Easy Diet Diary' smartphone app (Xyris Software, Highgate Hill QLD, Australia; https://connect.easydietdiary.com/ - version 5.0.22) was used to collect a 7-day food diary data from the participants. Participants were instructed to continue with their habitual dietary patterns and were advised on how to record all food items consumed

including the amounts, recipes and dietary supplements in the app. An Accredited Practising Dietitian was available to answer questions as needed throughout the 7-day food diary collection period. Nutrient data from consumed supplements were obtained from the nutritional information panels on the labels of the reported supplements and sent to the researchers as photographs. Dietary data was analysed using Foodworks professional software (Version 10, Xyris Software, QLD, Australia, 2012), using the AUSNUT 2011-13 food composition database [462]. Analysed nutrient data included nutrients consumed from both food and supplement sources. Where a food or beverage could not be identified in the AUSNUT 2011-13 database, an appropriate substitute was chosen using professional judgment by a dietitian. Substitutes were reviewed by a second Accredited Practising Dietitian to ensure quality and appropriateness of the substituted items. Supplement data was entered manually into Foodworks.

6.2.4.2 *Exercise training assessment*

A 7-day usual exercise training diary was collected to assess physical activity. The training diary was used to record the weekly duration (minutes) of exercise according to the mode of activity (for example, running) and the number of training sessions performed each week. Participants were asked to continue with their normal training regimens which did not include an 'off-season or taper period' as these individuals were already excluded. Training status was determined using De Pauw et al categorisations [463].

6.2.5 Dietary quality

Diet quality was assessed using the Alternate Healthy Eating Index 2010 (AHEI-2010). A modified version of the AHEI-2010 was implemented in this study, whereby the methodology described by Wang et al [305] was applied due to the dietary assessment data

being obtained via a food diary rather than a food frequency questionnaire as outlined in Study 1b. Briefly, the AHEI-2010 comprises of 11 components of which high intakes of six are considered desirable, four components where high intake is considered unfavourable and one component where moderate intake is best (alcohol). Each component was scored on a 10-point scale and subsequently summed to obtain the total AHEI-2010 score. Utilisation of the AHEI-2010 diet quality tool in Study 1b provided feasibility for the present study. Details of the scoring calculation are outlined in (**Table** 4.1).

6.2.6 Blood sample collection

A blood sample collection was performed using a finger prick method (OmegaQuant, South Dakota, United States), 30 minutes prior to the peak aerobic power test and, therefore, regarded as post prandial. Each blood spot was allowed to dry and sent to OmegaQuant Analytics, LLC for analysis. The dried blood spot method has been validated for measuring whole blood fatty acid profile and calculating the O3I of red blood cells (r=0.96). Detailed methods of the gas chromatography technique used in this study have been previously described [464]. O3I was used to classify cardiac risk (high risk, <4%; intermediate risk, 4% to 8%; and low risk, >8%) [465].

6.2.7 Statistical analysis

Analysis of the data was conducted using Graphpad (PRISM 8, La Jolla, CA, USA) software. Group differences, for characteristics (such as aerobic capacity, training), dietary intake and whole blood fatty acids / O3I, were analysed using unpaired t-tests for parametric data and Mann-Whitney tests for non-parametric data. The data was expressed as mean and 95% confidence intervals (CI) for parametric outcome measures and median and 25-75 percentiles for non-parametric data. Spearman r and Pearson r correlations were used to test for relationships between dietary intakes and whole blood FA status. Correlations were explored

as dietary groups and combined for the cohort due to the small sample size. Alpha was set at $P<0.05$.

6.3 Results

6.3.1 Participants

Twelve males adhering to a vegan diet and eight males adhering to an omnivorous diet completed the trial. Both groups were 'trained' endurance athletes as per De Pauw et al [463] (VO_{2max} mL · min^{-1} · kg^{-1} 55.0–64.9). No significant differences between the groups were observed with respect to age, weight, peak aerobic power or weekly time spent in usual training. The athletes in the vegan group had a significantly lower BMI (p=0.040) compared to the omnivorous group (**Table 6.1**).

Table 6.1. Anthropometric and training status characteristics.

	Omnivorous	Vegan	P value
Age, years	35.63 (30.43-40.82)	33.42 (29.57-37.26)	0.439
Weight, kg ^	77.70 (74.40-86.6)	78.25 (67.65-81.18)	0.665
BMI, kg/m^2	24.67 (23.03-26.27)	22.57 (21.20-23.95)	0.040
Peak VO_2, mL/kg/min	55.73 (50.90-60.55)	56.43 (51.98-60.89)	0.816
Peak VO_2, L/min	4.426 (4.007-4.845)	4.241 (3.844-4.637)	0.492
Weekly training time, minutes	381.4 (235.1-527.6)	340.4 (217.2-463.5)	0.636

Data expressed as mean and 95% CI.
^ Data expressed as median (25-75 percentile)
VO_2 - Volume of oxygen consumed.

6.3.2 Nutrient intakes

The vegan group consumed significantly less protein, total fat, saturated fat, monounsaturated fat, trans fat, cholesterol and LC n-3 PUFA (EPA, DPA and DHA) whilst consuming more fibre compared to the omnivorous group (**Table 6.2**) (dietary data inclusive of both food and supplement sources). As a percentage of energy, the vegan group obtained significantly more energy from available carbohydrates (49.3% vs 41.5%, p = 0.007), significantly less energy from protein (19.0% vs 14.2%, p = 0.0005) and less energy from fat (29.3% vs 33.8%. p = 0.058). The mean total energy intake was 13264kJ (omnivorous group) and 10727kJ (vegan group) although differences between groups did not statistically differ (p = 0.052) (**Table 6.2**). Two of the vegan participants in this trial were using an algal omega-3 supplement, whereas no participants in the omnivorous group were using a fish or algal omega-3 supplement. Intakes of sodium, calcium and iodine were significantly lower in the vegan group compared with the omnivorous group whereas beta-carotene was higher in the vegan group.

Table 6.2. Dietary intake characteristics from the omnivorous (n=8) and vegan (n=12) groups using a 7-day food diary.

	Omnivorous group	Vegan group	P value
Energy Total (kJ)	13264 (10464-16064)	10727 (9380-12074)	0.052
% Energy Carbohydrate	41.4 (37.2-45.5)	49.3 (45.3-52.8)	0.007
% Energy Protein	19.0 (16.0-22.0)	14.2 (13.2-15.1)	0.001
% Energy Fat	33.8 (30.5-37.1)	29.32 (26.0-32.7)	0.058
Carbohydrate (g)	329 (279-379)	321.5 (273.5-369.4)	0.813
g • CHO^{-1} • kg^{-1}	4.2 (3.4-5.0)	4.3 (3.6-5.0)	0.825
Protein (g) ^	129.3 (115.6-188.5)	82.1 (73.1-101.0)	0.003
g.• Pro^{-1} • kg^{-1} ^	1.6 (1.3-2.5)	1.0 (1.0-1.3)	0.010
Fat Total (g)	122.2 (90.3-154.1)	84.1 (72.9-95.2)	0.007
Saturated Fat (g)	43.7 (33.7-53.8)	18.4 (15.6-21.3)	<0.001
Polyunsaturated Fat (g)	18.6 (14.1-23.1)	23.5 (18.5-28.5)	0.141
Monounsaturated Fat (g)	49.6 (33.2-66.0)	35.6 (30.7-40.6)	0.039
Trans Fat (g) ^	1.5 (1.25-2.2)	0.4 (0.3-1.1)	<0.001
Cholesterol (mg) ^	352.9 (236.3-579.8)	18.0 (12.2-42.4)	<0.001
Alcohol (g) ^	2.5 (0.0-17.7)	0.0 (0.0-7.2)	0.625
Dietary fibre (g) ^	34.3 (27.2-46.0)	58.9 (49.3-72.7)	0.003
Thiamin (mg)	2.3 (1.3-3.3)	2.9 (1.3-4.6)	0.526
Riboflavin (mg) ^	3.0 (1.9-4.0)	1.5 (1.1-3.4)	0.098
Niacin (mg)	35.5 (25.4-45.6)	29.2 (24.3-34.1)	0.169
Vitamin C (mg)	158.5 (59.7-257.3)	256.7 (131.3-383.2)	0.224
Vitamin E (mg)	21.6 (13.3-29.9)	25.8 (20.2-31.4)	0.332
Vitamin B12 (ug) ^	6.1 (5.0-7.2)	211.5 (1.5-677.9)	0.238
Folate (ug)	735 (538-932)	1140 (813-1468)	0.053
Beta Carotene (ug)^	3664 (1191-6447)	11367 (4477-16622)	0.039
Sodium (mg)	3447 (2261-4633)	2306 (1844-2767)	0.029
Potassium (mg)	5245 (3554-6936)	5555 (4684-6426)	0.686
Magnesium (mg)	602 (447-757)	750 (637-864)	0.090
Calcium (mg)	1541 (1109-1973)	974 (797-1150)	0.005
Iron (mg) ^	16.0 (13.3-26.3)	19.9 (17.6-26.6)	0.157
Zinc (mg) ^	15.7 (12.8-17.5)	12.87 (10.6-19.8)	0.571
Iodine (ug)	263.8 (223.2-304.4)	146.4 (108.0-187.7)	<0.001
Selenium (ug)	130.1 (75.70-184.6)	67.3 (55.1-79.5)	0.005
Linoleic (g)	15.4 (11.3-20.3)	20.8 (17.0-24.5)	0.075
Alpha-linolenic ALA (g) ^	1.9 (1.4-2.6)	2.3 (2.0-4.9)	0.115
Eicosapentaenoic EPA (mg) ^	72.6 (43.2-162.8)	2.9 (0.6-26.8)	0.007
Docosapentaenoic DPA (mg) ^	81.0 (58.8-97.8)	6.5 (2.6-10.9)	0.001
Docosahexaenoic DHA (mg) ^	111.6 (85.8-543.1)	1.4 (0.8-10.6)	0.006

Data expressed as mean (95% CI). ^ Data expressed as median (25-75 percentile). P values calculated with unpaired t-tests for parametric data and Mann-Whitney tests for non-parametric data.

6.3.3 Whole blood and red blood cell fatty acids

Differences between dietary groups for saturated, monounsaturated and polyunsaturated whole blood fatty acids were observed (**Table 6.3**). Of particular relevance, the vegan group had higher linoleic (C18:2n-6) and lower gamma-linolenic (C18:3n-6), EPA (C20:5n-3) and DHA (C22:6n-3) ($P<0.05$) measures. This resulted in the whole blood n-6:n-3 and AA (Arachidonic acid):EPA ratios being significantly higher in the vegan group (**Table 6.3**). Both dietary groups had inadequate LC n-3 PUFA intakes resulting in O3I below recommendations. The vegan group exhibited a significantly lower O3I compared to the omnivorous group ($P<0.05$) (**Table 6.3**). Notably, the unsaturation index was lower in the vegan group compared to the omnivorous group. The vegan group also had significantly higher alpha-linolenic (α-LA) concentrations compared with the omnivorous group.

Table 6.3. Whole blood fatty acid profile and red blood cell O3I for the omnivorous (n=8) and vegan (n=12) groups.

Fatty acid		Omnivorous group	Vegan group	P value
Myristic	14:0	0.10 (0.74-1.64)	0.60 (0.52-0.79)	0.0201
Palmitic	16:0	22.33 (21.20-23.46)	19.87 (19.03-20.71)	<0.001
Stearic	C18:0	11.32(10.67-11.96)	11.47(10.81-12.13)	0.72
Arachidic	C20:0	0.24(0.21-0.26)	0.26(0.23-0.29)	0.18
Behenic	C22:0	0.46(0.37-0.55)	0.46(0.41-0.51)	0.95
Lignoceric	C24:0	0.99(0.81-1.17)	1.01(0.86-1.15)	0.84
Sum of SFA^	-	36.33(35.41-37.37)	33.37(32.60-34.38)	<0.01
Palmitelaidic	C16:1n-7t	0.14 (0.13-0.17)	0.07(0.06-0.08)	<0.001
Palmitoleic	C16:1n-7	1.24(0.74-1.74)	0.65(0.50-0.80)	0.005
Elaidic	C18:1t	0.55(0.46-0.65)	0.26(0.22-0.30)	<0.001
Oleic	C18:1n-9	21.10(19.55-22.65)	21.79(20.57-23.01)	0.44
Eicosenoic	C20:1n-9	0.21(0.16-0.26)	0.32(0.27-0.36)	0.002
Nervonic ^	C24:1n-9	1.08(0.92-1.20)	1.12(0.92-1.29)	0.46
Sum of MUFA	-	24.30(22.50-26.11)	24.24(23.01-25.47)	0.94
Linoelaidic	C18:2n-6t	0.31(0.27-0.35)	0.22(0.20-0.25)	<0.001
Linoleic	C18:2n-6	20.64(18.77-22.52)	24.82(23.40-26.24)	<0.001
gamma-Linolenic	C18:3n-6	0.37(0.24-0.50)	0.24(0.20-0.27)	0.01
Eicosadienoic	C20:2n-6	0.31(0.27-0.35)	0.38(0.35-0.42)	0.01
Dihomo-g-linolenic	C20:3n-6	1.41(1.23-1.57	1.60(1.42-1.78)	0.13
Arachidonic	C20:4n-6	9.44(8.21-10.68)	8.63(7.94-9.32)	0.17
Docosatetraenoic	C22:4n-6	1.25(1.00-1.50)	1.38(1.26-1.49)	0.23
Docosapentaenoic n-6 ^	C22:5n-6	0.25(0.19-0.37)	0.20(0.17-0.23)	0.098
Sum of omega-6 PUFA^	-	33.08(32.20-36.09)	38.26(36.67-39.18)	0.02
alpha-Linolenic ^	C18:3n-3	0.58(0.48-0.63)	0.68(0.61-0.90)	0.047
Eicosapentaenoic (EPA)	C20:5n-3	0.87(0.61-1.14)	0.56(0.45-0.68)	0.01
Docosapentaenoic (DPA)	C22:5n-3	1.07(0.72-1.40)	1.08(0.99-1.17)	0.91
Docosahexaenoic (DHA)	C22:6n-3	2.70(2.08-3.33)	1.90(1.47-2.33)	0.02
Sum of omega-3 PUFA^	-	5.26(4.03-5.99)	4.25(3.78-4.40)	0.23
n-6 /n-3 ^	-	6.23 (5.78-8.42)	9.11 (8.60-9.86)	0.004
AA/EPA	-	11.61 (8.92-14.31)	16.31 (13.67-18.95)	0.01
Omega-3 Index	-	5.40(4.55-6.23)	4.13(3.52-4.73)	0.01
Trans Fat Index	-	0.86(0.74-0.98)	0.48(0.42-0.53)	<0.001

Data expressed as mean (95% CI)
^ Data expressed as median (25-75 percentile)
AA; Arachidonic acid, SFA; Saturated Fatty Acid, MUFA; Monounsaturated fatty acid, PUFA; Polyunsaturated Fatty Acid.

6.3.4 Relationship between dietary and blood fatty acid profiles

Moderate to strong positive correlations were observed when the groups were pooled (n=20) between dietary intake of both DHA and EPA and whole blood cell concentrations of DHA and EPA (**Table 6.4**). Similarly, there was a moderate positive correlation between the dietary DHA (R= 0.624; $p<0.01$) and EPA (R= 0.650; $p<0.01$) and O3I. Strong negative correlations were observed between dietary DHA, DPA and EPA and the AA:EPA ratio in the study cohort (n=20). Of relevance, dietary alpha- linolenic acid was not correlated with red blood cell EPA, DHA or omega-3 index. When correlations between the vegan dietary intake and whole and red blood cell fatty acid status were performed (n=12), many of the correlations observed in the pooled data set disappeared (n=20; **Table 6.4**). Notably, no relationships were identified between dietary α-LA and DHA, EPA or O3I in the vegan or omnivorous group separately (**Table 6.4**).

Table 6.4. Correlation between fatty acid dietary intake and whole blood fatty acid profiles in the combined group study population (n=20)

Combined

	WB Linoleic C18:2n-6	WB alpha-Linolenic C18:3n-3	WB Eicosapentaenoic C20:5n-3	WB Docosahexaenoic C22:6n-3	Omega-3 Index	Trans Fat Index	WB n-6	WB n-3	WB n-6/n-3	WB AA:EPA
Dietary total fat	-0.208	-0.302	0.226	0.356	0.312	0.400	-0.047	0.137	-0.274	-0.058
Dietary saturated fat	-0.386	-0.427	0.477*	0.496*	0.487*	0.620**	-0.262	0.317	-0.531*	-0.438
Dietary trans Fat	-0.493*	-0.492*	0.400	0.241	0.241	0.617**	-0.415	0.021	-0.361	-0.405
Dietary polyunsaturated fat	0.429	0.263	-0.215	-0.093	-0.159	-0.417	0.448*	0.035	0.209	0.352
Dietary monounsaturated fat	-0.185	-0.420	0.161	0.192	0.153	0.286	-0.051	0.054	-0.119	-0.003
Dietary linoleic	0.464*	0.081	-0.253	-0.104	-0.213	-0.469*	0.469*	0.066	0.205	0.526
Dietary alpha linolenic	0.686**	0.568**	-0.229	-0.051	-0.108	-0.559**	0.699***	0.155	0.335	0.353
Dietary EPA	-0.337	-0.293	0.802***	0.595**	0.650**	0.674***	-0.397	0.559**	-0.789***	-0.842***
Dietary DPA	-0.353	-0.346	0.798***	0.690***	0.716***	0.726***	-0.319	0.565**	-0.707***	-0.767***
Dietary DHA	-0.408	-0.430	0.749***	0.583**	0.624**	0.695***	-0.490*	0.432	-0.747***	-0.773***

Omnivorous

	WB Linoleic C18:2n-6	WB alpha-Linolenic C18:3n-3	WB Eicosapentaenoic C20:5n-3	WB Docosahexaenoic C22:6n-3	Omega-3 Index	Trans Fat Index	WB n-6 (median)	WB n-3	WB n-6/n-3	WB AA:EPA
Dietary total fat	0.386	0.007	-0.091	0.045	0.017	-0.385	0.245	0.308	0.028	0.182
Dietary saturated fat	0.617*	-0.049	0.033	0.227	0.193	-0.527	0.364	0.420	-0.077	0.048

Dietary Trans Fat	0.147	-0.378	0.098	-0.056	-0.063	-0.119	-0.070	-0.091	0.014	-0.091
Dietary Polyunsaturated fat	0.159	0.329	-0.160	-0.077	-0.097	-0.193	0.077	0.217	0.000	0.277
Dietary monounsaturated fat	0.259	-0.503	-0.020	0.059	0.044	-0.257	0.098	-0.056	0.322	0.066
Dietary linoleic	0.137	-0.070	-0.118	0.071	0.033	-0.184	-0.091	0.245	-0.098	0.294
Dietary alpha linolenic	0.587*	0.706*	-0.147	-0.014	-0.028	-0.427	0.510	0.329	0.238	0.140
Dietary EPA	0.252	0.028	0.678*	0.147	0.203	0.336	-0.063	0.371	-0.517	-0.783**
Dietary DPA	0.322	-0.161	0.608*	0.378	0.441	0.210	-0.035	0.280	-0.224	-0.608*
Dietary DHA	0.098	-0.490	0.671*	0.252	0.273	0.448	-0.147	0.175	-0.434	-0.671*

Vegan

	WB Linoleic C18:2n-6	WB alpha-Linolenic C18:3n-3	WB Eicosapentaenoic C20:5n-3	WB Docosahexaenoic C22:6n-3	Omega-3 Index	Trans Fat Index	WB n-6	WB n-3	WB n-6/n-3	WB AA/EPA
Dietary total fat	0.344	0.063	-0.434	0.126	-0.046	-0.195	0.494	-0.187	0.239	0.643
Dietary saturated fat	0.233	0.215	-0.429	0.063	-0.098	-0.147	0.315	-0.221	0.228	0.579
Dietary Trans Fat	-0.224	0.133	-0.349	-0.726*	-0.740*	0.148	-0.334	-0.648	0.572	0.272
Dietary Polyunsaturated fat	0.562	-0.002	-0.545	0.303	0.066	-0.459	0.500	-0.176	0.239	0.592
Dietary monounsaturated fat	0.318	-0.042	-0.357	0.096	-0.045	-0.128	0.558	-0.160	0.234	0.633
Dietary linoleic	0.514	-0.081	-0.541	0.220	-0.004	-0.352	0.528	-0.212	0.277	0.636
Dietary alpha linolenic	0.610	0.011	-0.464	0.403	0.179	-0.489	0.719*	-0.029	0.148	0.645
Dietary EPA	-0.221	0.337	0.765*	0.516	0.709*	0.130	-0.121	0.733*	-0.688	-0.593
Dietary DPA	-0.302	0.163	0.860**	0.423	0.663*	0.237	-0.091	0.692	-0.659	-0.622
Dietary DHA	0.085	0.665	0.396	0.746*	0.774*	-0.235	0.049	0.700	-0.635	-0.289

Table completed using Spearman r analysis for non-parametric data and Pearson r value analysis for parametric data.

AA, Arachidonic acid, DHA, Docosahexaenoic acid; WB, Whole blood; EPA. Eicosapentaenoic Acid; DPA, Docosapentaenoic acid;.

* $p<0.05$, ** $p<0.01$ *** $p<0.005$

6.3.5 Diet quality

The vegan group consumed significantly more servings of vegetables (7.50 vs 4.87, p = 0.007) and nuts and legumes (2.60 vs 1.04, p=0.047) per day, whilst consuming fewer servings of red/processed meats (0.0 vs 0.90, p<0.0001) per day (**Table 6.5**). Daily wholegrain consumption was higher in the vegan group compared to the omnivorous group (313.0g/day vs 159.4g/day, p=0.043) whereas LC n-3 PUFA (9.74mg/day vs 247.4mg/day, p<0.0001) and sodium intake (2306mg/day vs 3447mg/day, p=0.029) were lower. These disparities in dietary intake translated into significant differences in scoring of the components between groups when the AHEI scoring matrix was applied (**Table 6.5**). Subsequently, the diet quality was significantly higher in the vegan group compared to the omnivorous group (78.24 vs 68.27, p=0.008). No correlation was observed between diet quality scores and any of the inflammatory markers, although, dietary quality was inversely correlated with BMI (r -0.455, p = 0.044).

Table 6.5. Diet quality in the omnivorous (n=8) and vegan (n=12) groups.

Component	Omnivorous group	Vegan group	AHEI criteria for minimum score	AHEI criteria for maximum score	AHEI-2010 Score (Omnivorous)	AHEI-2010 Score (Vegan)
Vegetables (servings/day) ^	4.87 (2.12-5.78)	7.50 (6.19-11.76) **	0	≥5	9.72 (4.25-10.00)	10.00 (10.00-10.00) *
Fruit (servings/day)	1.54 (0.44-2.63)	2.62 (1.49-3.74)	0	≥4	3.80 (1.16-6.45)	5.99 (3.68-8.29)
Whole grains (g/day)	159.4 (77.05-241.8)	313.0 (198.0-428.0) *	0	90	9.38 (8.40-10.00)	10.00 (10.00-10.00)
Sugar-sweetened beverages and fruit juice (servings/day) ^	0.06 (0.00-1.32)	0.17 (0.01-0.57)	≥1	0	9.38 (0.00-9.99)	8.26 (4.25-9.86)
Nuts and legumes (servings/day) ^	1.04 (0.22-2.77)	2.60 (2.14-4.05) *	0	≥1	8.42 (2.175-10.00)	10.00 (10.00-10.00) *
Red/processed meat servings/day ^	0.90 (0.4051-1.23)	0.0 (0.0-0.0) ***	≥1.5	0	3.99 (1.80-7.30)	10.00 (10.00-10.00) ***
Trans fat (% of energy) ^	0.31 (0.186-0.51)	0.18 (0.08-0.37)	≥4	≤0.5	10.00 (9.42-10.00)	10.00 (10.00-10.00) *
Long-chain omega-3 fatty acids (DHA/EPA; mg/day) ^	247.4 (197.3-814.3)	9.74 (3.50-25.61) ***	0	250	9.52 (7.89-10.00)	0.39 (0.14-1.02) ***
PUFU (% of energy)	6.50 (5.16-7.85)	5.51 (4.21-6.82)	≤2	≥10	4.35 (2.41-6.28)	5.25 (3.68-6.81)
Sodium (mg/day)	3447 (2261-4633)	2306 (1844-2769) *	Highest decile	Lowest decile	3.61 (1.01-6.22)	5.98 (4.09-7.74)
Alcohol (drinks/day) ^	0.22 (0.00-1.39)	0.00 (0.00-0.62)	≥3.5	0.5-2.0	2.50 (2.50-8.13)	2.50 (2.50-2.50)
Total Score			0	110	***68.27*** *(62.08-74.47)*	***78.24*** *(73.56-82.92)* **

Measured using the Alternate Healthy Eating Index-2010 (AHEI-2010 – adapted to metric)

Data expressed as mean and 95% CI. ^ Data expressed as median (25-75 percentile). * p<0.05, ** p<0.01, *** p<0.001

6.4 Discussion

Endurance athletes experience high training volumes and accompanying physiological strain and thus must carefully plan their dietary intake to avoid deficiencies [466]. Significant differences in nutrient intake were observed between groups and translated into dissimilarities in whole blood fatty acid blood concentrations (palmitic, palmitelaidic, palmitoleic, elaidic, linoelaidic, linoleic, gamma-linolenic, eicosenoic, eicosadienoic, EPA, DHA, n-6:n-3 ratios, AA:EPA ratios, omega-3 index and trans-fat index) which could have implications from a health and exercise training perspective. Specifically, in this cohort of male trained endurance athletes, following either a vegan or omnivorous eating pattern, it was evident that the O3I was sub-optimal in both dietary groups. Several other significant differences in nutrient intake were observed when dietary patterns were compared.

6.4.1 Energy and macronutrients,

The significantly lower BMI that was observed in the vegan group may in part be explained by the lower total energy intake in this group. Additionally, vegan dietary patterns appear to increase the thermic effect of food [41, 467] which can account for approximately 10% of total energy expenditure [467]. Optimised BMI in those following a vegan dietary pattern for endurance performance is one described feature which may prove advantageous [107]. Notwithstanding, insufficient energy intake can also result in detrimental effects on performance [468]. Given vegan dietary patterns are typically low in fat with a high dietary fiber content [36, 469], traits that reduce energy density, endurance athletes adhering to vegan dietary pattern should pay particular attention to their total energy intake.

Both groups were modestly under consuming carbohydrates as the recommendation for training ~1hr/day (~5-7g CHO/kg of body weight/day^{-1}) [52]. Underconsumption of

carbohydrates in endurance athletes is not uncommon [145]. For example, in 116 non-elite endurance athletes only 45.7% of athletes achieved the suggested CHO requirements (≥6 g/kg body weight/day) [146]. In the present study the vegan group appeared to modestly under consume protein while the omnivorous group achieved the recommend intake for endurance athletes (Vegan 1.04 g pro/day^{-1} | omnivorous 1.636 g pro/day^{-1}) [50]. Protein intake in endurance athletes consuming a vegan-based diet should be a focus during dietary planning. In terms of fat intake, it is recommended that endurance athletes follow public health guidelines to ensure fat intake is sufficient [50]. This is generally <30% of total energy from fat, saturated fats <10% of total energy intake, trans-fats <1% of total energy intake and replacement of saturated fat (and trans-fats) with unsaturated fats [470, 471] where possible. The vegan group demonstrated greater alignment to these guidelines with significantly less trans-fat, saturated fat and under <30% total energy intake from fat compared with the omnivorous group.

6.4.2 Micronutrients

Significant differences in several micronutrients were observed between the groups in the present study. Dietary iodine intakes were lower in the vegan group although the mean intake met the EAR (vegan group 146.4 µg/day | EAR of 100 µg/day [472]) suggestive of low risk of iodine inadequacy in this group. Similarly, calcium intake differed between the groups but both groups exceeded the EAR for calcium at 840mg/day [472]. In the present study, the vegan group consumed a higher intake of iron compared to the omnivorous group which aligns with other research examining the intakes of iron in recreational runners following a vegan (18.4mg/day) or omnivorous diet (15.3 mg/day) [472]. To the authors' knowledge, this is the only study that has examined serum ferritin levels in endurance athletes consuming vegan or omnivorous dietary patterns with no differences observed between the groups.

Nonetheless, ferritin levels in non-athletic female populations have been shown to be lower in people following a vegan dietary pattern compared with those consuming an omnivorous pattern, particularly in premenopausal female [328]. Therefore, dietary iron intake should be specifically considered when planning a diet for premenopausal females engaging endurance training. This is especially pertinent from a performance perspective given iron's critical role in energy metabolism and oxygen transportation and the increased risk of sub-optimal iron status in endurance athletes [473] although this may be less of a concern for male athletes due to their lower dietary iron requirements. Future studies exploring vegan dietary patterns and female endurance athletes could examine dietary iron intake and ferritin concentrations, especially as the current study only investigated dietary iron intake in males. The elevated baseline dietary sodium intake observed in the omnivorous group is unlikely to have any influence on performance outcomes [329], albeit is unfavourable from a health perspective [474].

6.4.3 Diet quality

The elevated AHEI-2010 score observed in the athletes in the vegan group (78.24), compared with the omnivorous group (68.27) aligned with previous research that was conducted in otherwise healthy populations. As outlined in detail in Study 1b, a recent systematic review assessing dietary quality in those following vegetarian diets reported a higher overall dietary quality was found compared with non-vegetarians [33]. Generally, the vegetarian-based diets tended to more closely align with recommendations for total fruit, whole grains, plant protein, and sodium but were less likely to align to recommendations for refined grains and total "protein foods" [33]. In the current study, there was an inverse relationship between BMI and AHEI-2010 score, which has been demonstrated in other research [364], although not for the same inflammatory markers. As the participants in this trial were trained endurance athletes,

it is likely that their baseline inflammatory markers were low in both groups as the regular exercise would produce an anti-inflammatory effect [247].

6.4.4 Whole and red blood fatty acids

The O3I of the vegan group in this trial was 4.13%, whilst the omnivorous group was modestly elevated at 5.40% although far from ideal (>8%). This was not surprising, as the typical Western style diet is less than optimal in providing LC n-3 PUFA [475] and remains true for athletes. For instance, the mean O3I in winter sport endurance athletes was 4.97 ± 1·19% [459], the mean O3I in collegiate athletes representing 21 sports in the U.S was 4.33 ± 0.81% [476] and in a sample of collegiate football athletes the mean O3I was 4.4% ± 0.8% [477]. These studies highlight the importance of endurance athletes following both omnivourous and vegan dietary patterns ensure that their dietary LC n-3 PUFA intake is sufficient.

Engaging in regular exercise is known to induce many health benefits, although, some studies have suggested endurance atheletes may be more susceptible to coronary plaque volume compared to matched sedentry groups [478, 479]. Further, endurance athletes may have increased myocardial damage compared to sedentary individuals [480]. If this is the case, reducing cardiac risk in endurance athletes should be a priority. By increasing the O3I in individuals following a vegan-based diet, they may further enhance their already favourable CVD risk.

While direct performance outcomes of increased EPA+DHA have not been observed, there are promising physiological attributes which may influence it. For instance, increased intake of EPA+DHA has been shown to lower heart rate at a submaximal workload through enhanced stroke volume and cardiac output [481, 482]. Similarly, cardiac and skeletal muscle

has been found to utilise oxygen more efficiently whilst a faster return to resting heart rate post exercise has also been observed [481, 482]. Most recently a systematic review reported LC n-3 PUFA were able to influence an athlete's physiology by modulating skeletal muscle recovery, postexercise nitric oxide responses, biomarkers of lipid peroxidation, TNF-α production by immune cells, and cardiovascular dynamics in cyclists [483]. The results of the literature review should be interpreted with caution however, as in general, the study designs for determining the effects of n-3 were lacking in some critical areas, such as the exclusion of known fish eaters. Regardless, to blunt exercise induced stress stimulated by endurance exercise, increasing one's O3I may be beneficial.

One proposed pathway to optimise n-3 LC PUFA in vegan populations has been to consume an abundance of α-LA. The conversion rates of α-LA, a precursor to the longer chain omega-3 FA's DHA and EPA, is generally considered to be poor in humans [484], although it has been hypothesised that populations with long-term adherence to vegan dietary patterns may be better able to convert α-LA to LC n-3 PUFA compared to those following omnivorous dietary patterns [484]. Limited research has explored this notion. In this study, the vegan endurance athlete group had significantly lower O3I compared to the omnivorous group, despite higher (although not statistically different) α-LA dietary intakes. In this small population of vegan trained endurance athletes, there was no correlation between α-LA and omega-3 index, DHA, or EPA, suggestive of poor conversion rates. Consuming a favourable ratio of dietary omega–6 to omega–3 fatty acids, ideally ~4:1 respectively has been suggested for optimal conversion of α-LA to DHA and EPA [485]; although there is evidence in rat models, showing that this has little impact on improving n-3 concentrations in multiple tissues including erythrocyte and heart [486] and skeletal muscle membranes [294]. In the present study, the ratio of whole blood n-6 to n-3 between groups was significantly different

with the omnivorous group's median being 6.23 (5.78-8.42) compared to the vegan group of 9.11 (8.60-9.86) where the ideal is <4. Perhaps if the dietary intake of n-6:n-3 was more closely aligned with a 4:1 ratio, the conversion of α-LA to DHA/EPA and subsequently O3I may have been improved.

In this study, significantly higher ratios of AA:EPA were seen in the vegan athletes compared to omnivorous athletes, providing evidence that dietary intake of LC n-3 PUFA should be considered. Low AA:EPA ratios (<11) have also been associated with lower rates of CVD disease and have been shown to inhibit tumorigenesis among other inflammatory conditions [487, 488]. Despite the elevated AA:EPA observed in this study, the wider body of evidence suggests that vegan dietary patterns are associated with reduced risk of cancer [30] and reduced systemic inflammation [254]. There were no significant differences in AA between groups, suggesting this disparity was due to low dietary intake of LC n-3 PUFA and/or poor conversion of α-LA in the vegan group. Optimising the ratio of AA:EPA in vegan-based athletes may be an area for consideration to further enhance their favourable inflammation profiles [254] for those following vegan diets. Practically speaking, to optimise the AA:EPA ratio, intakes of oils, seeds, nuts and full fat soy products high in linoleic acids should be avoided, whilst high consumption of flaxseeds and/or hemp seeds in addition to a concentrated algal oil rich in EPA/DHA could be consumed [489].

Whilst some fatty acids such as LC n-3 PUFA can have anti-inflammatory properties, others such as saturated fatty acids can be pro-inflammatory [115, 117, 490]. In the present study, total saturated fat consumption was significantly lower in the vegan endurance athlete group compared to the omnivorous group which mirrors findings from non-athletic vegan- and omnivorous groups [491, 492]. Specifically in this study, myristic and palmitic saturated fatty

acids were both significantly higher in the omnivorous athlete group which has been suggested to raise total and LDL cholesterol concentrations, and may increase coagulation and insulin resistance [115]. Saturated fat is also correlated with many pro-inflammatory pathways including; IL-1, IL-6, CRP and TNFα [117, 118]. Chronic elevation of pro-inflammatory such as these have been shown to predict risk CVD [493], type 2 diabetes mellitus [494] some cancers [495] and to a small extent, may modulate cytokine production during and post exercise [496].

6.5 Limitations and strengths

Despite this study being one of the few to provide an in-depth analysis of fatty acids in trained individuals, there were some limitations. Due to the small sample size in both groups, results should be interpreted with caution, particularly the correlations. In addition, this research was limited to male athletes, meaning results may not be generalisable to female athletes. Further, as some food and beverages were not available in the AUSNUT 2011-13 food composition database, small differences in nutrient intakes in some foods may have resulted. It was also beyond the scope of this study design to link any of the dietary characteristics or whole blood fatty acids to endurance performance, for two reasons. Firstly, both groups were sub-optimal in their O3I and secondly, the study design was cross-sectional. There were several strengths to this study. Differences between groups (peak aerobic power, training duration/week) were matched, which has been overlooked in previous studies exploring dietary patterns in athletes. Most notably, this was the first study to investigate fatty acid profiles in a trained endurance population following a vegan eating pattern. Additionally, a detailed food and exercise diary was collected and analysed during a steady and consistent time of training.

6.6 Conclusion

To the candidate's knowledge, this is the first study to investigate whole blood fatty profiles and O3I in vegan and omnivorous groups of aerobically trained male endurance athletes matched for age, training volume and aerobic capacity. Several dietary components of the vegan diet group appeared desirable from a reduced cardiovascular risk perspective, including significantly lowered total fat, saturated fat, trans-fat, cholesterol and increased fibre intake compared to the omnivorous group. Further, in this study a vegan dietary pattern provided a high diet quality whilst providing sufficient nutrients required for the endurance athlete. However, the O3I in both groups was suboptimal with the vegan dietary group critically low with a mean O3I of 4.13%; with some individuals <4% compared to a mean of 5.40% in the omnivorous group. Increasing the dietary DHA and EPA intake in endurance-based populations following vegan dietary patterns may assist in reducing the physiological stress associated with endurance training whilst optimising the O3I.

As there were significant differences in diet quality and several nutrients of relevance to exercise, including carbohydrate, protein and omega-3, between endurance athletes following either a vegan or omnivorous dietary pattern in this study, it follows that there may also be differences in various aspects of exercise physiology. This concept has seldom been considered in the literature and therefore warrants investigation.

7 Physiological outcomes in age, sex and aerobically matched individuals consuming a vegan or omnivorous dietary pattern during steady state exercise conditions | Study 4.

7.1 Introduction

As vegetarian-based dietary patterns appear to be associated with reduced baseline inflammatory markers compared to omnivorous dietary patterns (study 2), it is reasonable to hypothesise that differences in cytokine production during endurance exercise may also exist between groups with different dietary patterns. Additionally, diet quality and nutrient status appear to be disparate between dietary groups as seen in study 3, which may influence various exercise related physiology. From an exercise perspective, the cardiovascular physiology observed in people who follow a vegan dietary pattern may favourably influence endurance performance. For instance, the consumption of a vegan dietary pattern can optimise body composition [32], may reduce blood viscosity via reduced plasma lipid concentrations [34], improve atrial compliance, and improve endothelial function [151, 152] which has been hypothesised to improve vascular flow and tissue oxygenation [107].

Vegan dietary patterns are also typically rich in 'non-nutritive' compounds such as polyphenols and carotenoids, [14, 497] which have been shown to beneficially influence the immune system [79, 498] reduce upper respiratory tract infections [83] and have been linked to reduced systemic inflammation [82] which may provide flow on effects for exercise physiology. These factors have scarcely been investigated in the literature.

It was, therefore, the aim of this study to investigate if differences exist between physiological outcomes i.e., cardiometabolic parameters and inflammatory biomarkers in a group of aerobically, age and sex matched individuals consuming vegan or omnivorous dietary patterns during clamped steady state exercise conditions.

7.2 Materials and Methods

7.2.1 Study design

This study used the same sample of male endurance trained athletes following either a habitual vegan or non-vegan dietary pattern from study 3. Additional measures reported here ensure that the study achieved its aims. The recruitment process was outlined in Study 3 with participants required to attend a laboratory at the University of Wollongong on three occasions (**Figure 7.1**). Visit 1 involved a familiarization and maximal aerobic power testing. At the end of the first laboratory visit an Accrediting Practising Dietitian (JC) provided instructions about how to complete a 7-day food diary as described in Chapter 6. During the second laboratory visit a baseline venous blood sample was collected and participants performed exercise testing during walking, and then clamped conditions of 60% and 90% of their maximal aerobic power. After their second laboratory visit, participants were asked to record their overnight heart rate using a wearable electrocardiograph (ECG) eFaros device (eFaros, Bittium Corporation, Finland). During laboratory visits 1 and 2, central and peripheral cardiopulmonary and metabolic profiles were also collected. The final visit was 24 hours after the exercise testing for a second blood sample. On each occasion, body mass was measured for eligible participants using anthropometric scales in duplicate (SECA875 flat scale, SECA, United Kingdom) and height was measured with a stadiometer (SECA217 Stable Stadiometer, SECA, United Kingdom) under laboratory conditions whilst in light clothing and without shoes. Six skinfold thickness measurements were obtained (medial calf, anterior thigh, abdominal, subscapular, triceps, and supra-spinale: Eiken skinfold calliper, Meikosha, Tokyo, Japan). Two measurements from each site were averaged, unless they differed by more than 5 mm, in which case a third replicate performed, with the mode then used as performed in Bowes et al 2021 [499].

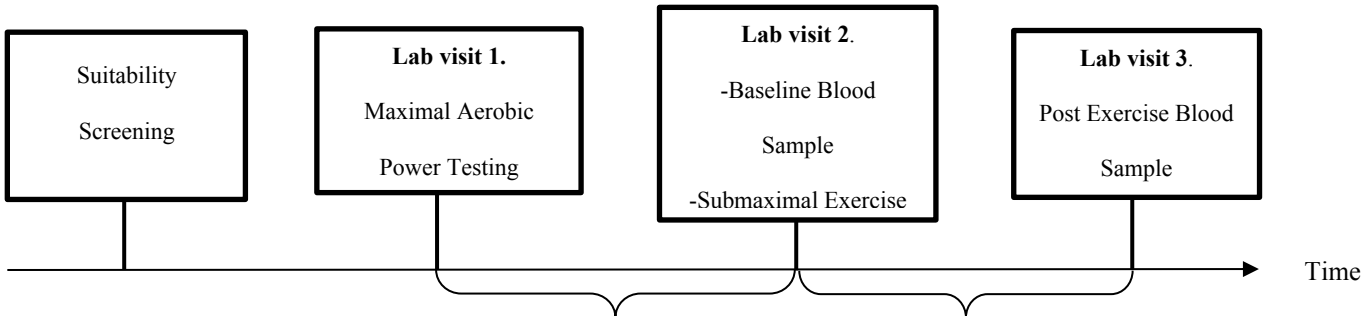


Figure 7.1. Conceptual framework of the study design

7.2.2 Maximal aerobic power

On laboratory visit 1, participants were seated for ten minutes whilst blood pressure and expired gases were collected. Participants then completed an incremental ramp running protocol on a treadmill (Cosmos 65, h/p/cosmos sports and medical GmbH, Germany) to attain maximal aerobic power. All participants started with five minutes of walking at 4 km/hr. From there, the treadmill speed was increased to 8 km/h (gradient 1%) and then further increased by 1 km/h each minute until a rated perceived exertion of 14 on a 15 category Borg scale (6-20) was achieved [500]. At this stage the treadmill speed was maintained, and the gradient was increased by 1% every minute until voluntary exhaustion was achieved. The highest oxygen consumption over a 30 second period was recorded as the peak (L/min).

7.2.3 Submaximal exercise

Upon arrival for laboratory visit 2, participants similarly were seated for ten minutes whilst blood pressure and expired gases were collected. The submaximal exercise testing involved three distinct states. Participants commenced with walking for five minutes at 4 km/hr. Upon completion, the treadmill speed was increased to elicit a submaximal running intensity of ~60% of their maximal aerobic power and this state was clamped for a duration of 40 minutes. The treadmill speed was modulated to ensure participants remained at ~60% of their

maximal aerobic power. Before commencing the final state (90% of maximal aerobic power) participants completed a further eight minutes of walking at 4 km/hr. The final state was then completed at 90% of maximal aerobic power for a duration of eight minutes. Most importantly, speed and/or gradient was modulated to maintain participants in a clamped state. The exercise protocol was selected based on a previous study demonstrating elevated IL-6 following a similar running protocol [252]. Exercise testing for maximal aerobic power and submaximal exercise was conducted in a temperature-controlled laboratory (21-23 degrees Celsius with 40-50% relative humidity) during a testing window spanning from 08:00 – 17:00. Participants were asked to refrain from consuming alcohol in the 24 hours leading up to both exercise tests. Participants were instructed to continue to follow their habitual dietary pattern which included the consumption of food up until two hours prior to the testing and to prepare for the peak aerobic power test and submaximal exercise as if they were completing an interval session akin to their normal training regime.

7.2.4 Blood sampling and analysis

Blood collection was performed by a trained phlebotomist who collected two samples, one at baseline during lab visit two and one 24 hours post exercise (laboratory visit 3). For both blood tests a sample was placed on ice and transferred to a quality assured pathology laboratory (Southern IML Pathology Australia) who performed a Full Blood Count analysis while the other sample was centrifuged at 2000 x g for 10min to separate the serum which was then transferred to Eppendorf tubes in 0.5mL aliquots and stored at -80^{o}C for later analysis. The serum was analysed for the concentration of CRP (ab99995), Human IgA (ab196263) and Fibrinogen (ab208036) in duplicate by ELISA Kits (Abcam, Cambridge, UK), as per the manufacturer's instructions. Briefly, the serum was diluted (in assay buffer using the following dilution factors: Fibrinogen, 1:50,000, CRP, 1:10,000, and IgA,

1:50,000), and provided standards and serum were added to the wells containing capture antibody and incubated for 2.5 hours at room temperature with shaking (400 rpm). The wells were washed with wash solution (phosphate buffered saline (PBS) containing Tween-20 detergent), biotinylated secondary antibody added, and incubated for 1 hour at RT with shaking (400 rpm). The wells were washed with wash solution, and horse radish peroxidase (HRP)-streptavidin (SA) added and incubated for 45 minutes at room temperature with shaking (400 rpm). The solution was discarded, tetramethylbenzidine (TMB) substrate added for 30 minutes at room temperature in the dark (cover with foil) with shaking (400 rpm), and stop solution (1 M H_2SO_4) added. Absorbance (450 and 570 nm) was measured using a SpectraMax Plus 384 (Molecular Devices, Sunnyvale, CA, USA). Inflammatory Cytokine concentrations were measured using a human T helper-1 LEGENDPlex kit (741036, BioLegend, San Diego, CA, USA) as per the manufacturer's instructions. Briefly, serum was diluted (1:2 in assay buffer), and provided standards, serum, and beads were added to wells and incubated for two hours at room temperature in the dark (cover with foil) with shaking (800 rpm) on an orbital shaker. The plate was centrifuged (250 xg, 5 minutes at room temperature), solutions were discarded, and the plate washed with wash buffer (provided) by centrifugation (250 xg, 5 minutes, RT) and the solution discarded. Detection antibodies were added to each well and incubated for one hour at RT in the dark (cover with foil) with shaking (800 rpm). Streptavidin (SA) conjugated-phycoerythrin (PE) beads were added to each well and incubated for 30 minutes at RT in the dark (cover with foil) with shaking (800 rpm) on an orbital shaker. The plate was centrifuged (250 xg, 5 minutes, RT), the solutions were discarded, and the plate was washed with wash buffer (provided) by centrifugation (250 xg, 5 minutes, RT) and the solution discarded. Wash buffer (provided) was added to wells, the beads were resuspended by repeated pipetting, and analysed using an Attune NxT flow cytometer with autosampler (Life Technologies, Waltham, MA, USA), using filters [561]

585/16 for PE, and [638] 670/14 for allophycocyanin (APC).

7.2.5 Oxygen consumption

Breath by breath expired gases were collected using a two-way breathing valve (Hans Rudolph, Shawnee, USA) and analyzed using a calibrated gas analysis system (TrueOne 2400, Parvo Medics, USA). On each occasion volume was calibrated using a 3.012 L syringe and oxygen and carbon dioxide was calibrated using two-point line of room air and standard gas (oxygen: 15.94% carbon dioxide: 4.03%).

7.2.6 Heart rate

Continuous heart rate was collected during the maximal aerobic power testing and submaximal exercise testing (60% & 90% PeakVO_2) exercise trials using a Polar heart rate monitor (Polar 800cx, Polar, Finland). For overnight heart rate, a wearable electrocardiograph (ECG) device (eFaros, Bittium Corporation, Finland) was fitted (lead I). All heart rate analyses were performed using Kubios HRV software version 3.4.1. Analysis of sleep, sitting, walking, 60% of peak aerobic power, 90% of peak aerobic power, and maximal peak aerobic power heart rate data were calculated from an intermediate 6-hour, 5-minute, 4-minute, 30-minute, five minute and a 30 second block of data, respectively. Relative heart rate percentages were calculated using the Karvonen equation [501].

Equation 1. Relative heart rate percentage (Karvonen equation)

*(1) Relative HR% = Mean HR for intensity – sleep HR / Max HR – sleep HR)*100*

Analysis of heart rate variability was also performed in Kubios and included data from time (mean RR, SDNN and RMSSD), frequency (power ms2 LF, HF and LF/HF ratio, power nu LF and HF) and nonlinear (SD1 SD2, SD2/SD1 ratio, DFA α1 and DFA α2) domains.

7.2.7 Muscle oxygen saturation

Analysis of muscle oxygen saturation (SmO_2) was analysed using a near-infrared spectroscopy device (Moxy, Fortiori Design LLC, USA) placed on the vastus laterals muscle. SmO_2 was recorded continuously during both laboratory visits with data extraction performed at sitting, 60% of peak aerobic power, 90% of peak aerobic power, and maximal peak aerobic power from a 5-minute, 30-minute, five minute and a 30 second block of data, respectively.

7.2.8 Substrate utilisation

Carbohydrate and fat oxidation (g/min^{-1}) were computed at walking and 60% of maximal aerobic power via indirect calorimetry using stoichiometric equations [502].

Equation 2 and 3. Carbohydrate (2) and fat (3) oxidisation (g/min^{-1}).

(2) $4.585*VCO_2 - 3.226*VO_2$

(3) $1.695*VO_2 - 1.701*VCO_2$

Protein oxidation was assumed to be negligible as per substrate oxidation during exercise protocols, whilst lactate production and lactate clearance were assumed to be coupled at these intensities [503].

7.2.9 Statistical analysis

The Noordzij et al guidelines [504] were used to determine the sample size with the primary marker, hs-IL-6 modelled from the Almada et al study [252] that assessed hs-IL-6 post exercise. Using this, it was determined that six participants in each dietary pattern group were required for a 80% power. Analysis of the collected data was conducted using Graphpad (PRISM 8, La Jolla, CA, USA) software. Heart rate data was transcribed into Statistix10 (Analytical Software, Tallahassee, FL, USA) for statistical analysis. Group differences were

analysed using independent samples t-test for parametric data and Mann-Whitney for non-parametric baseline data. The data collected was expressed as mean and 95% confidence intervals (CI) for parametric outcome measures and median and interquartile ranges for non-parametric data. Two-way ANOVA analyses were applied for repeated measures to test for between groups differences with Bonferroni post hoc analysis performed. Correlations were explored between dietary quality scores and anthropometric and inflammatory markers. Alpha was set at $P<0.05$.

7.3 Results

7.3.1 Participants

Twenty male endurance athletes completed the study (twelve from the vegan and eight to the omnivorous dietary pattern group). No significant differences between the groups were observed with respect to age, weight, sum of the 6-point skin fold measurements, blood pressure, peak aerobic power or weekly time spent training. The athletes who were following a vegan dietary pattern had a significantly lower BMI ($p=0.0401$) compared to the athletes who were following an omnivorous dietary pattern (**Table** 7.1), herein referred to as the vegan and omnivorous groups. The self-reported mean diet duration in the vegan group was 3.9 years (1.76-6.03 95% C.I) whereas the omnivorous group had followed their diet for their entire lives.

Table 7.1. Anthropometric and training status characteristics

Study group	Omnivorous	Vegan	P value
Age, years	35.63 (30.43-40.82)	33.42 (29.57-37.26)	0.439
Weight, kg ^	77.70 (74.40-86.6)	78.25 (67.65-81.18)	0.664
BMI, kg/m^2	24.67 (23.03-26.27)	22.57 (21.20-23.95)	0.040
Skinfold (Sum of 6 points - mm)	79.31 (37.89-120.7)	62.74 (56.69-68.79)	0.2122
Blood Pressure (Systolic)	125.0 (117.5-132.5)	124.9 (120.8-129.0)	0.981
Blood Pressure (Diastolic)	74.50 (71.07-77.93)	73.17 (69.52-76.81)	0.5785
Peak VO_2, mL/kg/min	55.73 (50.90-60.55)	56.43 (51.98-60.89)	0.816
Peak VO_2, L/min	4.426 (4.007-4.845)	4.241 (3.844-4.637)	0.492
Weekly training time, minutes	381.4 (235.1-527.6)	340.4 (217.2-463.5)	0.636

Data expressed as mean and 95% CI. ^ Data expressed as median (25-75 percentile), VO_2 - Volume of oxygen consumed. P-value determined using independent samples t-test for parametric data and Mann-Whitney for non-parametric baseline data.

7.3.2 Cardiometabolic measures

Heart rate, oxygen consumption and muscle oxygen saturation

Absolute mean HR was comparable between groups for each condition (overnight HR, sitting HR, walking HR, 60% MaxVO_2 HR, 90% MaxVO_2 HR and MaxVO_2 HR). Significantly different absolute mean HR's were observed for each condition ($p<0.0001$) although, not between sitting and walking ($p=0.961$) or 90% and Max HR ($p=0.921$). No differences in the relative HR percentages were observed between the groups at any exercise intensity (**Table** 7.2**)**. Both groups relative mean HR exceeded the externally set workload intensities of peak aerobic output percentage. At 60% of peak aerobic power the relative mean HR was 68.5% and 70.6% while at 90% of peak aerobic power the relative mean HR was 95.4% and 97% in the omnivorous and vegan groups, respectively. There were no differences in the absolute oxygen consumption (L/min) during any condition between the groups. Likewise, no significant differences between the groups were observed in oxygen consumption as measured by relative oxygen consumption as a percentage of seated, during walking, 60% or 90% maximum aerobic power. At maximal aerobic power there was a greater degree of oxygen muscle desaturation in the vegan group (11.17% vs 22.79% $p=0.041$) but not during

any other condition. The repeated measures ANOVA on muscle saturation by group was not significant, although was trending this way (p=0.053). The Pearson r test revealed a significant moderate correlation at $90\%_{peak}$ (r=0.562, p=0.024) and a strong correlation at $60\%_{peak}$ (r=0.688, p=0.007) aerobic power. Overall, there were no statistically significant differences to any domain of heart rate variability between groups for all exercise conditions (**Table** 7.3)

Table 7.2. Absolute HR and HR percentages and VO_2 and VO_2 percentages at varying intensities.

	HR [bpm] (SD)			**% Max HR (SD)**			**VO_2 [L.min] (SD)**			**%VO_2 of Max (SD)**			**SmO_2 [%] (SD)**		
	Omnivore	*Vegan*	*p*	*Omnivore*	*Vegan*	*p*	*Omnivore*	*Vegan*	*p*	*Omnivore*	*Vegan*	*p*	*Omnivore*	*Vegan*	*p*
Overnight	52.83(7.66)	48.12(4.97)	0.16												
Sitting	53.48(8.68)	53.55(8.40)	0.99	0.01 (7.33)	3.99(4.82)	0.16	0.30(0.04)	0.30(0.04)	0.99				70.09(7.34)	66.18(9.92)	0.36
Walking	72.51(8.59)	74.75(11.44)	0.64	15.06(3.84)	19.85(7.02)	0.10	0.88(0.07)	0.83(0.09)	0.17	14.34(2.09)	13.75(2.15)	0.55			
60%	141.50(16.41)	142.20(12.28)	0.92	68.55(6.02)	70.64(5.18)	0.42	2.72(0.27)	2.61(0.34)	0.42	58.89(2.14)	58.66(2.42)	0.83	57.82(20.76)	49.17(7.64)	0.27
90%	175.30(13.40)	177.00(10.10)	0.75	95.43(2.67)	97.00(3.95)	0.18	4.09(0.50)	3.85(0.53)	0.34	91.77(4.52)	90.32(2.76)	0.38	32.93(21.17)	21.45(6.32)	0.14
Peak	181.20(14.94)	181.20(11.21)	0.99				4.43(0.50)	4.24(0.63)	0.50				22.79(16.29)	11.17(5.66)	0.04

HR, Heart rate; bpm, beats per minute; SD, standard deviation; SmO_2, muscle oxygen saturation

Table 7.3. Heart rate variability between omnivorous and vegan groups for varying conditions.

	Exercise Type	Omnivorous	Vegan	P value
		Time		
Mean RR (ms)	Sleep	1156.6 (166.21)	1248.6 (114.37)	0.4939
	Sitting	1111.4 (185.28)	1136.2 (175.03)	
	Walking	805.32 (85.25)	828.26 (138.17)	
	60%	415.34 (44.97)	415.51 (31.8)	
	90%	335.64 (24.05)	333.87 (16.41)	
	Peak	334.79 (23.43)	328.64 (16.19)	
SDNN (ms)	Sleep	57.25 (19.75)	86.95 (37.92)	0.0793
	Sitting	60.35 (32.09)	68.01 (20.88)	
	Walking	44.36 (10.34)	38.26 (14.12)	
	60%	11.05 (17.85)	6.23 (8.51)	
	90%	7.28 (11.84)	4.32 (5.5)	
	Peak	2.72 (0.8)	2.94 (0.8)	
		Non-Linear		
SD2:SD1 Ratio	Sleep	1.88 (0.34)	1.55 (0.26)	0.0769
	Sitting	1.62 (0.42)	1.58 (0.43)	
	Walking	2.72 (0.35)	2.12 (0.58)	
	60%	1.62 (0.39)	1.25 (0.31)	
	90%	1.03 (0.31)	0.96 (0.28)	
	Peak	0.74 (0.15)	0.87 (0.25)	
DFA α1	Sleep	1.17 (0.14)	0.96 (0.18)	0.2918
	Sitting	1 (0.2)	0.96 (0.23)	
	Walking	1.43 (0.16)	1.18 (0.23)	
	60%	0.75 (0.19)	0.64 (0.19)	
	90%	0.61 (0.16)	0.57 (0.24)	
	Peak	0.42 (0.19)	0.44 (0.24)	

SDNN: Standard deviation of NN intervals, DFA α1: Detrended fluctuation analysis, SD2: Poincaré plot standard deviation along the line of identity, SD1: Poincaré plot standard deviation perpendicular the line of identity.

7.3.3 Metabolism

No differences were observed between the groups when a two-way ANOVA was applied for total fats (p=0.581) or carbohydrate (p=0.943) substrate utilisation at walking or 60% peak VO_2 (**Table** 7.4).

Table 7.4. Substrate utilisation at various intensities from omnivorous (n=8) and vegan (n=12) groups.

	Vegan	95% CI	Omnivorous	95% CI	p value
Carbohydrate (g/min^{-1})					
Walking	0.3454	0.215-0.476	0.2152	-0.003-0.434	0.228
60% peak VO2	2.476	2.139-2.813	2.332	1.785-2.880	0.734
Fat (gmin^{-1})					
Walking	0.2861	0.238-0.334	0.3604	0.292-0.429	0.051
60% peak VO2	0.3796	0.253-0.507	0.492	0.311-0.673	0.248

p values calculated using independent samples t tests.

7.3.4 Inflammation

No differences in CRP, fibrinogen, IgA, were observed between either group or within groups at baseline or post-test (**Table** 7.5**).** Total red blood cell count and haemoglobin were significantly different between the groups at pre- and post-test between groups ($p<0.05$). Similarly, haematocrit was significantly different between groups post exercise ($p<0.05$). No differences in the white blood cells (p=0.422) or platelets (p=0.059) were observed within or between the groups.

Table 7.5. Baseline inflammatory, immune, and full blood count markers between omnivorous and vegan groups.

	Omnivorous			**Vegan**			
	Baseline (95% C.I)	*Post (95% C.I)*	*Mean change*	*Baseline (95% C.I)*	*Post (95% C.I)*	*Mean change (95% C.I)*	*p-value (time x group)*
CRP (g/L)	0.683(0.47-0.90)	0.71(0.46-0.96)	0.026(-0.08-0.14)	0.659(0.40-0.92)	0.711(0.46-0.97)	0.052(-0.04-0.15)	0.670
Fibrinogen (g/L)	1.97(1.50-2.45)	1.88(1.39-2.36)	-0.099(-0.85-0.65)	2.31(1.66-2.95)	2.12(1.41-2.82)	-0.19(-0.82 to 0.45)	0.827
IL-2 (pg/ml) ^	2.54(-2.15-7.22)	2.53(-2.53-7.59)	-0.004(-0.51-0.50)	0.681(0.07-1.29)	0.764(-0.05-1.58)	0.08(-0.37 to 0.53)	0.754
IL-6 (pg/ml) ^	2.59(-1.32-6.49)	2.7(-0.91-6.31)	0.11(-1.27 to 1.49)	1.05(0.19-1.78)	1.41(0.47-2.09)	0.36(-0.87-1.59)	0.742
IL-10 (pg/ml) ^	1.57(-0.284-3.43)	0.90(-0.73-2.52)	-0.679(-1.86-0.50)	0.616(-0.52-1.75)	0.907(-0.37-2.18)	0.291(-0.76 to 1.35)	0.150
IFN-g (pg/ml) ^	9.54(2.62-16.5)	7.61(1.59-13.60)	-1.93(-6.35-2.49)	4.08(3.04-5.13)	4.44(1.87-7.00)	0.35(-3.42-4.12)	0.349
TNF-a (pg/ml) ^	8.77(-0.967-18.5)	7.53(-1.40-16.50)	-1.23(-4.68-2.21)	3.30(1.02-5.58)	3.69(0.14-7.23)	0.383(-2.56-3.32)	0.394
IgA (ng/ml)	1.76(1.00-2.51)	1.91(1.41-2.40)	0.132(-0.45-0.72)	2.03(1.65-2.41)	1.99(1.42-2.57)	-0.03(-0.51-0.44)	0.592
Haemoglobin (g/L)	152(145-159) [a]	151(144-159) [a]	-0.875(-5.05-3.30)	144(139-149) [b]	142(138-147) [b]	-2.36(-6.26-1.54)	0.528
Red Blood cell count (10^{12}/L)	5.06(4.84-5.29) [a]	5.10(4.87-5.33) [c]	0.038(-0.11 -0.19)	4.75(4.59-4.91) [a]	4.67(4.55-4.78) [c]	-0.07(-0.21-0.07)	0.207
Haematocrit	0.451(0.43-0.47)	0.46(0.44-0.48) [a]	0.007(-0.00-0.02)	0.433(0.42-0.45)	0.429(0.42-0.44) [a]	-0.007(-0.02-0.01)	0.089
White Cell Count (10^9/L)	6.13(4.70-7.55)	6.41(5.05-7.78)	0.288(-0.53-1.10)	5.35(4.40-6.31)	5.30(4.24-6.36)	-0.082(-0.84-0.68)	0.422
Neutrophils (10^9/L)	3.33(2.22-4.43)	3.35(2.42-4.28)	-0.008(-0.75-0.74)	2.95(2.19-3.72)	2.90(1.97-3.83)	0.025(-0.78 to 0.83)	0.941
Lymphocytes (10^9/L)	2.11(1.71-2.52)	2.34(1.90-2.77) [a]	0.225(-0.09 to 0.55)	1.82(1.51-2.12)	1.77(1.47-2.06) [a]	-0.092(-0.39 to 0.21)	0.091
Monocytes (10^9/L)	0.550(0.44-0.66)	0.525(0.39-0.66)	-0.025(-0.11-0.06)	0.445(0.36-0.53)	0.478(0.41-0.54)	0.017(-0.06-0.10)	0.383
Eosinophils (10^9/L)	0.113(0.08-0.14)	0.12(0.09-0.16)	0.00(-0.03-0.03)	0.149(0.08-0.21)	0.149(0.08-0.21)	0.004(-0.03-0.03)	0.812
Platelets (10^9/L)	240(180-300)	256(198-314)	15.9(4.34-27.4)	262(234-291)	263(231-295)	2.83(-8.04-13.7)	0.059

'a', 'b' indicates significant differences between groups ($p<0.05$), c reflects $p<0.01$ between group

7.4 Discussion

The current study has explored metabolism, cardiometabolic and cardiovascular parameters and inflammatory biomarkers in multiple clamped states from rest to peak exercise in endurance athletes who were consuming a vegan or omnivorous dietary pattern. In summary, this study has revealed that, despite the differences in dietary intake, there were largely no physiological differences between dietary groups. However, there was some indication, at least for these conditions of clamped metabolic work rate, that muscle oxygen tissue saturation is lowered in the case of athletes following vegan diets, especially at the point of exhaustion.

7.4.1 Cardiovascular and cardiometabolic measures

In the current study no differences were observed in oxygen consumption at walking, 60% Peak VO_2 or 90% Peak VO_2 which was expected due to the clamped nature of the trial. Although, in a recent study examining the effects of vegan diets and physical activity consumption of a vegan diet was shown to invoke significantly higher submaximal endurance time to exhaustion (12.2 ± 5.7 vs. 8.8 ± 3.0 min; $p = 0.007$) at 70% MaxVO_2 compared with consumption of an omnivorous diet in active individuals [149]. Similarly, in a randomized parallel study comparing the consumption of a whole food plant based diet (n=37) with a conventional diet for diabetes management (n=37) in patients with type 2 diabetes for 12 weeks, consumption of the whole food plant based diet induced significant increases in relative peak VO_2 and maximum watt output, whereas the conventional diet did not [505]. Weight loss, a common characteristic associated with whole food plant-based diets may have contributed to the increase in relative peak VO_2, however. Notably, in the aforementioned study the participants had type 2 diabetes, a chronic disease associated with systemic inflammation making it difficult to compare to the healthy trained males in the present study.

It has previously been hypothesized that the most substantial improvements in exercise physiology or performance outcomes elicited by the consumption of a vegan diet may indeed be observed during extended endurance activities, or multi stage events as these are likely to induce substantial multi system stress comparable to the stresses associated with T2DM [496, 506].

On the contrary, Nebl et al examined arterial lactate and glucose concentrations finding no differences between vegetarian, vegan or omnivorous dietary groups when a peak exercise test was performed in recreational runners matched for training time, frequency, distance and maximum power output [147]. Hietavala et al similarly examined oxygen consumption at submaximal intensities between recreationally active men consuming a mostly vegan (limited dairy was incorporated) diet and a omnivorous diet [507] and found increased oxygen consumption in those consuming the vegan diet 40%, 60% and 80% of VO_{2Max} which is indicative of poorer exercise economy. Substantial limitations with this study exist however, most notably only nine participants were involved in the trial with the prescribed dietary pattern followed for only four days. Further, the prescribed diet was PRAL (potential renal acid load) optimized consisting of mostly fruits and vegetables, described as a low-protein diet. Other studies have compared maximal aerobic power between groups consuming a vegan and omnivorous diet [148], although have not explored the exercise efficiency parameters, an important outcome to determine if true performance outcomes exist between dietary groups.

As haemoglobin, haemocrit and RBC counts all influence oxygen carrying capacity, and oxygen carrying capacity directly correlates to aerobic performance [508], the significantly lowered counts of these blood components in the vegan group in this study may have

considerable implications on aerobic performance. Evidence of this was apparent in the muscle oxygen saturation values whereby the vegan group exhibited consistently lowered muscle saturation across each exercise intensity whilst at peak aerobic power there was a significant desaturation in the vegan group. Iron and vitamin B12 metabolism may be linked to the lowered concentrations of observed haemoglobin, haemotocrit and RBC's as both nutrients have crucial roles in erythropoiesis [509] and have been noted to be harder to obtain on a vegan diet [43, 328]. Athletes are generally at a greater risk for iron depletion due to exercise induced iron losses [327], and in vegetarian populations serum ferritin concentrations may also be reduced [328]. Similarly, those following vegetarian-based diets must regularly consume foods fortified with vitamin B12 or specifically supplement the vitamin as few plant sources contain traces of B-12 [44, 291]. From Study 1b it could be seen that collectively only 58% of self-reported recreational, competitive and professional respondents following vegetarian-based diets reported supplementing vitamin B12 which may help explain the lowered RBC counts in part. The relationship between haemoglobin, haemotocrit and RBC's and aerobic capacity in endurance athletes following vegan populations warrants additional investigation.

Regular exercise and consumption of n-3 both share outcomes of reduction of heart disease and an ability to modify heart rate and heart rate variability [345]. Individuals who increase their exercise have been shown to have statistically significant differences in their heart rate variability. This is particularly the case for the standard deviation of NN (SDNN) intervals, the root mean square of successive differences between normal heartbeats, absolute power of the low-frequency band (0.04–0.15 Hz), absolute power of the high-frequency band (0.15–0.4 Hz), poincaré plot standard deviation perpendicular the line of identity and poincaré plot standard deviation along the line of identity [510]. Notably, a plateau effect has been

described from the influence of physical activity on heart rate variability [510]. This plateau may in part explain why no differences in HRV were observed in this study as weekly exercise duration and maximum aerobic power were matched and both groups were engaging in regular activity. To our knowledge, this is the first study to evaluate HRV in endurance athletes consuming a vegan dietary pattern. No significant differences in any of the observed HRV domains were observed, which mirror the findings in non-endurance-based participants consuming a vegetarian diet (consuming dairy) [511]. In contrast, higher 24 hour SDNN but lower day-time HRV and shorter daytime inter-beat intervals in were observed in BMI matched middle aged participants consuming a vegan dietary pattern, relative to comparable individuals consuming an omnivorous diet [512].

7.4.2 Metabolism

Endurance performance can be largely dictated by endogenous carbohydrate availability [52, 513]. It has been hypothesized that consuming a vegan diet might be ideal for optimising glycogen stores due to the rich composition of carbohydrate foods [107] which may subsequently improve performance by delaying fatigue and/or performing a greater amount of work in a shorter timeframe [144]. In the present study, the vegan group did obtain a higher amount of energy as a percentage of total energy from carbohydrates whilst lower protein and fat, although the differences did not translate to a difference in substrate utilization. Fat utilization approached significance between the groups during walking ($p=0.051$) with the omnivorous group using it to a greater degree. It may be that the translation of macronutrient intakes to substrate utilization may be more pronounced in activities of a longer duration than was performed in the present study which may have a performance advantage by delaying the onset of fatigue due to increased muscle and liver glycogen stores [514].

7.4.3 Inflammation

In this study of male aerobically matched endurance athletes there were no significant differences in baseline inflammatory or immunological markers between groups. This is in contrast to the systematic review and meta-analysis conducted in chapter 3 showing that consuming a vegetarian-based diet is associated with a favourable inflammatory and immunological profile [254]. This is most likely explained by both groups in the current study engaging in regular aerobic activity and following dietary patterns of a high quality as measured by the AHEI-2010. No significant differences in fibrinogen, CRP, interleukins, IgA or leukocytes were observed within groups pre-post exercise suggestive of insufficient exercise induced stress to invoke a measurable inflammatory response 24 hours post exercise.

Although an elevated post exercise inflammatory or immunological response was not induced in this pilot study, differences in the nutrients reflect conceivable distinctions in biomarkers post exercise between the groups. For instance, saturated fat intake was significantly higher in the omnivorous group and has been correlated with proinflammatory markers such as CRP, TNF-α, IL-1 and IL-6 [115, 117, 118]. Vegan dietary patterns inherently omit dietary cholesterol due to the exclusion of animal based products, as was observed in this trial, and has been linked to pro-inflammatory pathways [515]. Fibre intake was significantly higher in the vegan group which is associated with reduced CVD risk and CRP levels [516]. Further, there may be observable differences in IL-6 concentrations post endurance exercise between dietary groups where there are substantial differences in carbohydrate intake as reduced muscle glycogen is a stimulus for IL-6 production [517]. Beta-carotene was significantly elevated in the vegan group compared to the omnivorous group which is inversely associated with CRP and IL-6 [518] and has been noted to influence immune function [141, 379]. Conversely, LC n-3 PUFA were lower in the vegan group which have been linked to lowered

systemic inflammatory markers [519] and have been described with relevance to exercise performance and cardiac risk in Chapter 5.

Due to the significant differences observed in some key nutrients between dietary groups, it remains plausible that small differences in the inflammatory responses may exist between dietary groups. The consumption of vegan diets have been used therapeutically and have the most profound effect in settings of substantial physiological stress [7, 15]. Therefore, sustained endurance events or multi-stage races might provide an opportunity to investigate the potential differences in these biomarkers as these events also induce multi-system physiological stress including the endocrine and immune systems [520].

7.5 Limitations and strengths

Considering the small sample size in this study, results of this trial should be interpreted with caution. The study was performed with male endurance athletes and is, therefore, not generalisable to female endurance athletes. Similarly, the participants were not fixed to a particular sport i.e. triathlon, but participated in a range of sports increasing the heterogeneity. On the other hand, the study was matched for age, maximal aerobic power, weekly training volume and gender to eliminate many confounding variables, providing an ideal lens to examine the potential differences in physiology between the groups. Matching of these variables has been overlooked in previous studies [148] which could lead to disingenuous inferences, particularly about performance outcomes. Additionally, this study has analysed the dietary data of both groups in terms of diet quality with a validated tool finding both groups consumed a diet of high quality. It is widely accepted that well planned vegan diets are health promoting but comparing a healthful vegan dietary pattern to a substandard Western style diet or conversely a poorly planned vegan diet to a high quality omnivorous could also lead to skewed results.

7.6 Conclusion

This study builds on the findings of the previous studies reported in this thesis by examining whether the differences in nutrient intakes reported in a larger sample of self-reported athletes in Study 1b and observed in a specific group of endurance athletes in Study 3 translated into any physiological differences between omnivorous and vegan dietary patterns. In contrast to the evidence from the broader literature explored in Study 2, inflammatory and immune biomarkers were comparable between the dietary groups, although, a more prolonged exercise duration may have induced a more pronounced response required to observe potential differences. Future studies examining the influence of vegan dietary patterns on inflammatory markers should consider both sexes, longer exercise durations, possibly over several days to substantially evoke inflammatory markers whilst collecting blood samples frequently. Notably however, RBC, haemoglobin and haemotocrit levels were identified to be disparate between groups which appeared to translate into greater muscle desaturation, particularly at peak aerobic power in the vegan group which warrants further investigation. Mean heart rate, heart rate variability and oxygen consumption were all comparable at each exercise intensity. In this study carbohydrate intakes were modestly elevated in the athletes adhering to a vegan dietary pattern, although substrate utilisation did not differ between the groups.

8 Conclusions and future directions

8.1 Thesis summary

Vegetarian-based dietary patterns are observed to have a protective effect against many conditions, yet limited research had previously examined the inflammatory and immune effects of this diet which could provide some mechanistic understandings into these protective effects. Likewise, athletes are also increasingly embracing vegetarian-based diets yet there is scarce evidence exploring the various exercise related physiological effects a diet of this nature might provide. It was, therefore, the aim of this thesis to explore differences in dietary intake in individuals following vegetarian-based or omnivorous dietary patterns, and to determine if dietary intakes translate into dissimilarities in inflammatory and immune biomarkers. A secondary aim was to determine if the dietary intakes between those following vegetarian-based or omnivorous dietary patterns could influence diet and nutrient quality and subsequently modulate exercise related physiology.

This thesis has provided an overarching assessment of inflammatory and immune profiles in those following vegetarian-based diets whilst exploring nutrient status, diet quality and supplementation patterns in individuals following a vegetarian-based dietary patterns. This body of work provides insights into potential effects and relationships between the vegetarian-based dietary pattern and ways in which it may ameliorate inflammation. By examining these areas this thesis has contributed to the body of evidence by further establishing a framework to guide those following a vegetarian-based diets. Whilst there has been much theoretical and anecdotal suggestion of a vegetarian-based diet's ability to optimise endurance performance, limited objective data existed prior to this work. This thesis has empirically compared physiologically relevant exercise markers after an acute bout of exercise between those following vegetarian-based and omnivorous dietary patterns finding no differences in healthy trained endurance athletes matched for age, training volume and

maximal aerobic capacity. These findings may assist endurance athletes and the general population who choose to follow vegetarian-based diets make informed decisions regarding their nutritional intake to assist with their exercise performance.

8.2 Core thesis findings and summary

As highlighted in Chapter 1, vegetarian-based dietary patterns are associated with improvements and protection against many chronic diseases which has largely been attributed to their food components including fruits, vegetables, nuts, seeds and legumes and absence of animal foods. However, research examining the synergistic effects of these foods consumed within a vegetarian-based dietary context on inflammatory biomarkers, and exercise physiology was lacking. In particular, limited research was available on the characteristics of athletes following vegetarian based diets their reasons for following these diets.

A cross sectional online survey (study 1a) was implemented to explore dietary behaviors, supplementation patterns and motives in this population group. Here, it was found that almost 25% of athletes were adopting a vegetarian-based diet with the aspiration of improving their exercise performance whilst sports supplements with known and accepted ergogenic effects were seldomly used and could be an area for consideration for athletes following vegetarian-based diets. This study was used to inform Studies 1b, 3 and 4 by providing an understanding into the behaviours and motives of these athletes.

In study 1b it was found that athletes following a vegetarian-based diet with the aspiration of enhancing performance appeared to have improved diet quality when compared to those athletes who were following a vegetarian-based diet for other reasons. The nutrient analysis suggested that a vegetarian-based dietary pattern could provide a high diet quality for athletes to support physical activity. Although, intake of some nutrients in this vegetarian-based

population may have been insufficient including vitamin B12 and LC n3 PUFA's. Identifying LC n3 PUFA dietary intake may be sub-optimal in these athletes informed by study 3 where whole and red blood cell fatty acid profiles were assessed. As studies 1a and 1b were conducted online, comprised solely of athletes following vegetarian-based diets, a study was required to test the hypothesis that because of the varied dietary intake between those following vegetarian and non-vegetarian dietary patterns there may also be differences in various biomarkers including inflammatory and immunomarkers.

A systematic review and meta-analysis was performed to determine the relationship between vegetarian-based dietary patterns and inflammatory and immune markers (Study 2). Those following vegetarian-based dietary patterns were found to have significantly lower concentrations of CRP, fibrinogen and total leukocytes compared to those following non-vegetarian dietary patterns in observational studies. Limited studies were available to perform a quantitative analysis in the form of a meta-analysis for many markers of interest in both observational and intervention studies (including all interleukins, TNF-α, NK cell activity, lymphocytes, neutrophils, monocytes, eosinophils, basophils, IgG, IgA, IgD, IgE and IgM) yet, all markers analysed appeared to be favorable in the vegetarian-based group in qualitative syntheses. Since markers of inflammation and immune function were observed to be disparate between those following a vegetarian, compared to non-vegetarian diet in the systematic review, it was plausible that these changes might produce modest reductions in the inflammatory response during endurance performance whilst also impacting upon other physiological responses, however there was limited published research conducted in athlete populations. A laboratory study was, therefore, required to substantiate the findings from the previous studies in this thesis and explore potential differences in exercise physiology between a group of athletes following a vegetarian-based and non-vegetarian based diet.

To empirically assess the nutritional composition and diet quality in a group of vegan and non-vegan trained endurance athletes a laboratory study was implemented (Study 3). The vegan group consumed significantly less protein, total, saturated, monounsaturated and trans fats, and cholesterol whilst consuming more dietary fibre compared to the omnivorous group. As a percentage of total energy, the vegan group consumed significantly more energy from carbohydrates (49.3% vs 41.5%, $p = 0.0068$), significantly less energy from protein (19.0% vs 14.2%, $p<0.001$ and less energy from fat (29.3% vs 33.8%. $p = 0.0583$). As dietary LC n3 PUFA's intake was observed to be low in the online survey (Chapter 1b), intake of LC n3 PUFA's were also focus of this laboratory study and whole blood serum analysis was analysed. The O3I in both groups was suboptimal with the vegan dietary group critically low with a mean O3I of 4.13%; with some individuals <4% compared to a mean of 5.40% in the omnivorous group.

The laboratory study was also performed to enable physiological markers to be examined in a controlled setting building on the findings of the online survey (Study 1a and 1b) and systematic review (Study 3). Mean heart rate, heart rate variability and oxygen consumption were all comparable at each exercise intensity. It had previously been hypothesised that a vegan dietary pattern may be suited to optimising glycogen stores due to the rich composition of carbohydrate foods typically consumed on a diet of this nature. In this study carbohydrate intakes were modestly elevated in the athletes adhering to a vegan dietary pattern, although substrate utilisation did not differ between the groups. Inflammatory and immune biomarkers were comparable between the dietary groups, although, a more prolonged exercise duration may have induced a more discernable response required to observe potential differences. Importantly, this study did identify baseline RBC's, hemoglobin and hematocrit to be

significantly lower in the vegan group which appeared to increase muscle desaturation particularly at peak aerobic power.

8.3 Limitations

The specific limitations of each study have been outlined throughout this thesis in the corresponding chapters, however, it is important to consider the overarching limitations.

Firstly, this thesis has considered vegetarian-based dietary patterns to be collectively characterised by a majority of energy from dietary intake being obtained from plants. In reality, there are considerable disparities between nutrient intakes and the various vegetarian-based dietary patterns. This is evident in this thesis as Studies 1a, 1b and 2 included participants who may have followed one of many of the sub-classes of vegetarian-based dietary patterns (i.e. ovo-lacto-vegetarian, ovo-vegetarian, vegan) whereas Studies 3 and 4 were exclusive vegan. Differences between these dietary patterns are likely to exist.

Secondly, the generalisability of the findings from this thesis are limited across the studies performed. In the systematic review, there was substantial variation between the population demographics. While studies included in the review came from a range of continents including Asia, Africa, North America, South America and Europe there were limited sample sizes across the included studies. Given that within these continents individual countries have different cuisines and cultures, the generalisability to apply the findings broadly is currently limited. In the exercise trial, only a small pool of male endurance trained participants were included. The findings, therefore, cannot be applied female athletes. Further, in the exercise trial participants were not fixed to a particular endurance modality and differences between sports i.e. triathlon, running etc. cannot be excluded. Similarly, respondents from the online

survey engaged in an array of sports and physical activity modalities and were from a large range of countries. Therefore, the generalisability of the results from this study should not be uniformly applied across all population groups following vegetarian-based diets or across all exercise modalities (i.e., weightlifting, sprinting, gymnastics).

Thirdly, the results in thesis may have been confounded by lifestyle factors other than diet. Individuals following a vegetarian-based diet are more likely to engage in healthy lifestyle behaviours such as yoga/meditation and avoidance of alcohol and smoking which can have positive health outcomes [521, 522]. Whilst care was taken to eliminate or control these factors, in some instances this was difficult. For instance, several studies in the systematic review did not report on lifestyle behaviours such as smoking, alcohol consumption or meditative practices.

Finally, all studies in this thesis were prone to under-reporting by participants including the studies utilised in the SR. It has been suggested that under-reporting by participants is a fundamental barrier to collecting accurate dietary data with the 18-54% of participants potentially under-reporting [523]. Care was taken to avoid this limitation by using a validated 24 hour recall in study 1b and a rigorous explanation of the importance of accurate diet data collection in Study 3 with detailed instructions on how to record intake as well as ongoing availability between participants and an accredited practicing dietitian, but nonetheless, remains a limitation.

8.4 Future directions and recommendation

1. **A focus on inflammatory and immunological outcomes in plant-based diets**

Study 2 of this thesis outlined several significant differences in inflammatory markers between vegetarian and non-vegetarian-based dietary patterns. However, due to the limited

number of studies, quantitative analysis was not possible for many biomarkers in both observational and intervention studies including interleukins (all), TNF-α, NK cell activity, lymphocytes, neutrophils, monocytes, eosinophils, basophils, IgG, IgA, IgD, IgE and IgM. Future research should concentrate on investigating potential differences in these biomarkers with a particular focus on immune biomarkers and function between dietary groups given the encouraging, but limited findings of this systematic review which included lowered total leukocyte and lymphocyte (in 2/4 studies) concentrations in addition to improved NK cell activity in 1/2 studies in vegetarian-based groups. Since it appears inflammatory profiles may be favourable in those following vegetarian-based populations it is plausible immune function may also be improved given the inherent link between the two physiological systems. Emerging research has shown preliminary evidence of this concept with protection against COVID-19 [524].

2. Energy and macronutrient considerations for endurance-based athletes following vegetarian-based dietary patterns.

The significantly lower BMI that was observed in the vegan group in study 3 is characteristic among those following vegetarian-based dietary patterns [525]. Interestingly, an optimised BMI in those following a vegan dietary pattern for endurance performance is one feature which has been suggested to be advantageous to endurance athletes owing to the increased efficiency. Although, insufficient energy intake can result in detrimental effects on performance [468]. In study 3 the vegan dietary group consumed less energy than the non-vegan group. Given these results, and that vegan dietary patterns are typically low in fat with a high dietary fiber content [36, 469], traits that reduce energy density, endurance athletes adhering to vegan dietary patterns should pay particular attention to their total energy intake,

potentially increasing it.

3. **Protein considerations for endurance-based athletes following vegetarian-based dietary patterns.**

Protein requirements are increased for the athletes engaging in regular physical activity with ~1.4g/kg suggested for endurance athletes depending on the intensity and volume [50, 359]. This thesis has suggested that those following vegetarian-based dietary patterns, and particularly vegan dietary patterns may be modestly under consuming protein. Sufficient protein is easily attainable on a vegan dietary pattern, although, like any diet selected to fuel exercise performance, careful consideration should be given to dietary planning. Adequate protein intake in endurance athletes will optimise the facilitation the skeletal muscle adaptive response and will improve muscle reconditioning.

4. **Increasing dietary long-chain omega-3 fatty acid intake**.

Study 3 data indicated dietary omega-3 intake including alpha-linolenic acid may be low in athletes choosing to follow a vegetarian-based dietary pattern which similarly aligns with other population data. To the candidate's knowledge, no research has evaluated whole blood fatty acid profiles in endurance athletes following a vegan based dietary pattern however and was, therefore, a focus for Study 3. In both vegan and non-vegan groups O3I was suboptimal with the vegan dietary group critically low with a mean O3I of 4.13% compared to a mean of 5.40% in the non-vegan group. Further research should be conducted in both male and female vegan population groups with a larger study sample, to determine if optimising the dietary n-6:n-3 ratio (ideally <4:1) is associated with an increased O3I. Given the low O3I results

observed in this study in both dietary groups, and the clear association of the blood fatty profile with dietary intake, athletes more broadly, but especially athletes following vegan eating patterns, should carefully consider their dietary intake of LC n-3 PUFA. Increasing the dietary DHA and EPA intake in endurance-based populations following vegan dietary patterns may assist in reducing the physiological stress associated with endurance training whilst optimising the O3I.

5. **Increased exercise intensity and duration to examine possible differences in inflammatory and physiologically relevant exercise physiology between individuals following vegetarian and non-vegetarian dietary patterns.**

Much of the evidence concerning the effect of vegetarian-based diets to favourably modulate biomarkers relevant to health has been observed in populations with chronic disease, with elevated inflammatory biomarkers. As a result, positive changes to the diet in these disease states delivers clinically significant and measurable health outcomes. Conversely, the small changes which may exist for healthy endurance trained athletes, following vegetarian-based diets, may be less pronounced and difficult to detect given the subtle changes to these systems and normalised biomarkers in healthy athletes.

Translation of any physiological relevant biomarkers to improved endurance performance will be particularly challenging to quantify, as exercise endurance testing is inherently difficult to measure with an array of co-variables such as sleep, stress, training load, training history, motivation, psychology etc. It would be reasonable to hypothesise that perhaps the largest physiological exercise relevant effect of following a vegetarian-based diet may be observed in the context of multi-stage or ultra-endurance events. Ultra-endurance activities

are known to induce multi-systems physiological stress akin to being in 'diseased state'. Since vegetarian-based dietary patterns tend to have their most profound effects in states of considerable stress [7, 15] the recovery phase of an ultra-endurance event might provide the best opportunity for benefits to be observed. Therefore, future research investigating the effects of diet to modulate physiological, particularly inflammation and immune function could consider doing so in an ultra-endurance multi-stage context. Drawing blood immediately post exercise and at several time points thereafter up until 24 hours post exercise to capture key biomarkers such as IL-1 IL-10, TNF-a, CRP, leukocytes and fibrinogen which can vary in their peak concentrations post exercise is also recommended.

6. Further considerations and research examining the relationships between a vegetarian-based dietary pattern and muscle saturation, RBC's haemoglobin and haemotocrit, iron and vitamin B12.

Study 4 demonstrated that compared to the non-vegan dietary group, the vegan group exhibited significantly lowered RBC's heamoglobin and haemotocrit which appeared to modulate oxygen saturation as the vegan group had significantly increased muscle desaturation at peak aerobic power. It is well known that these blood components all influence oxygen carrying capacity which directly correlates to aerobic performance [508]. The findings indicate a poorer oxygen carrying capacity for athletes choosing to follow a vegetarian-based diet but further investigation is required to substantiate these findings. Research should focus on the relationship between muscle saturation and RBC's, haemotocrit, and haemoglobin whilst monitoring dietary intake and supplementation of vitamin B12 and iron which have crucial roles in erythropoiesis [509]. Endurance athletes following vegetarian based diets should carefully consider their nutrient intake and in

particular their iron and vitamin B12 consumption to ensure their nutritional demands to meet the demands of their physical activity. Vitamin B12 supplementation is strongly recommended.

8.5 Final remark

In summary, the findings from this thesis partly support the central hypothesis that 'due to differences in nutrient composition intake between vegetarian and non-vegetarian-based dietary patterns, disparities will exist in various biomarkers between groups, which will influence exercise physiology, particularly during endurance exercise'. Vegetarian-based dietary patterns provided differing nutrient intake compositions compared to non-vegetarian-based dietary patterns which favourably modulated various inflammatory related biomarkers such as CRP and fibrinogen in the general population. No relevant inflammatory differences were observed between healthy athletes following either a vegetarian or non-vegetarian-based diet prior to, or post an acute bout of endurance activity, however. There may be some physiological differences in RBC's and their components between dietary groups which is known to modulate oxygen carrying capacity. Further research should be conducted to substantiate this.

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10 Appendices

Appendix A. Vegetarian-based dietary behaviours and physical activity survey

Vegetarian-based dietary behaviours and physical activity

Demographics

What is your gender?

Male

Female

Other

I am (check one):

□ ***18 – 19 years***

□ ***20 - 29 years***

□ ***30 - 39 years***

□ ***40 - 49 years***

□ ***50 - 59 years***

□ ***60 - 69 years***

□ ***70 years or older***

What is your country of origin? (Drop down box)

What is your current Zip/Postal Code? _____

What is your Race/Ethnicity:

□ White/Caucasian

□ Aboriginal or Torres Strait Islander

□ African American

□ American Indian/Native American

□ Asian

□ Other (please specify)

What is your height?

_____ cm

OR

_____ feet _____ Inches

OR

□ Unsure

What is your body weight?

_____ kg

OR

_____ Stones _____ Pounds

OR

□ Unsure

What is the highest level of education you have completed or enrolled in?

□ Primary School

□ I completed/am enrolled high school

□ I completed/am enrolled in Diploma or equivalent

□ University (I completed/am enrolled in a bachelor/undergraduate degree)

□ University (I completed/am enrolled in a honours/master's degree)

□ University (I completed/am enrolled in a doctoral degree)

Have you ever completed any formal studies in human nutrition?

□ Yes

□ No

If yes, please list the formal studies you have completed in human nutrition

Physical activity

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time (IPAQ-SF).

During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ *days per week*

□ *No vigorous physical activities (Skip to question X)*

How much time did you <u>usually</u> spend doing vigorous physical activities on one of those days?

_____ *hours per day*

_____ *minutes per day*

□ *Don't know/Not sure*

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breath somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ *days per week*

□ *No moderate physical activities (Skip to question X)*

How much time did you <u>usually</u> spend doing moderate physical activities on one of those days?

_____ *hours per day*

_____ *minutes per day*

□ *Don't know/Not sure*

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ *days per week*

□ *No walking (Skip to question X)*

How much time did you <u>usually</u> spend walking on one of those days?

_____ *hours per day*

_____ *minutes per day*

□ *Don't know/Not sure*

This question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the last 7 days, how much time did you spend sitting on a weekday?

_____ *hours per day*

_____ *minutes per day*

□ *Don't know/Not sure*

During the past 12 months, what is the primary type of physical activity you participated in? (please select only one option)

Please note, there is a subsequent question which asks about other types of physical activity you engage in – This question is concerned with your primary/main physical activity.

I did not participate in any physical activity
Swimming
Cycling
Jogging
Triathlon
Walking
Weight Lifting
Team sport (ie football, netball, rugby union, basketball etc)
Dancing
Athletics
Surfing
Yoga
Skiing/Snowboarding
Fitness classes
Personal training
Tennis
Other (Please describe)

_____

Approximately how many months and years have you participated in this type activity?

_____ *Months* _____ *Years*

In the last 12 months, how many times per week <u>on average</u> do you participate in this activity?

_____ sessions / week

How do you describe your current involvement in this activity?

Recreational (participate to keep fit/healthy and for enjoyment)

Competitive (participate to keep fit and healthy but have a somewhat structured training program with the aim to compete/win events/competitions)

Professional (Get paid to compete and/or have sponsors related to your sport)

During the past 12 months, what <u>other types</u> of physical activity did you participate in? (more than one option can be selected)

I did not participate in any physical activity

Swimming

Cycling

Jogging

Triathlon

Walking

Weight Lifting

Team sport (ie football, netball, rugby union, basketball etc)

Dancing

Athletics

Surfing

Yoga

Skiing/Snowboarding

Fitness classes

Personal training

Tennis

Other (Please describe)

_____

Approximately how many months and years have you participated in this type activity?

_____ Months _____ Years

In the last 12 months, how many times per week on average do you participate in this activity?

_____ sessions / week

(Duration and times/week relevant for each activity selected by participant)

General eating pattern

I never eat (check all, if any, that apply):

- □ Red meat
- □ Poultry (chicken, turkey, duck, etc.)
- □ Fish/seafood
- □ Dairy products
- □ Eggs
- □ Honey
- □ I eat all of the above
- □ Don't know

My eating pattern can be referred to as (choose one only)?

Omnivorous (Consume meat at least 2 times/week)

Vegan (exclude all meat and animal products)

Ovo-Vegetarian (exclude all meat and milk/cheese/butter products but consume eggs)

Lacto-Vegetarian (exclude all meat and egg products but consume milk/cheese/butter products)

Ovo-Lacto-Vegetarian (exclude all meat but consume egg, milk, cheese and butter products)

Pescetarian (exclude all land animals but consume marine products)

Other (please expand)

For how long have you followed this eating pattern? If you are unsure of months, please round up to the nearest year.

Years Months

The main reason I follow this eating pattern is because of (check only one):

- □ I have always eaten this way
- □ Health
- □ Animal rights
- □ Improve my performance
- □ Weight loss
- □ The environment
- □ My religion or spiritual beliefs
- □ My Family and/or friends do and it's easy for me
- □ Other _____

Do you <u>purposely</u> follow a specific type of diet? (check all, if any, that apply):

- □ No
- □ Yes (Please select below; more than one box may be selected)
    - □ High carbohydrate
    - □ High Fat
    - □ High Protein
    - □ Low Carbohydrate
    - □ Low Fat
    - □ Low Protein
    - □ Other

Do you purposely <u>restrict or eliminate</u> food types to improve your participation in physical activity?

- □ Yes (please comment on the types of restrictions or foods you eliminate)
- □ No

Are there any foods or food groups you <u>specifically include</u> in your eating pattern to improve your physical activity?

- □ Yes (please comment on the types of foods you include)

□ No

Do you currently monitor your energy intake (kilojoules or calories) to improve your physical activity?

Yes

No

Do you purposely restrict energy intake to improve your physical activity?

Yes

No

How important is your overall eating pattern and/or specific components of your intake in terms of the following? 1= not important 5 = extremely important

	1	2	3	4	5
Preparation for physical activity					
Recovery from physical activity					
Hydration status					
Increased body weight					
Decreased body weight					
Body composition					
Body fat					
Delayed muscle soreness					
Muscular fatigue					
Muscular power					
Muscular strength					
Endurance capacity					

<u>Supplementation</u>

This section asks you about any supplements you currently consume to maximise your athletic performance. These may be taken as an individual product (capsule or tablet) or may be contained in a mixed product such as a sports drink or energy bar.

If you currently use a supplement not included on this list, the final option will allow you to enter any others.

In the past 12 months, have you used supplements of any form?

NOTE - Supplements may include: *components of sport drinks, bars and gels, vitamins, minerals, carbohydrate, protein and essential fatty acid concentrates, meal replacement products, herbal preparations, amino acids, enzymes, organ tissues, glandulars, metabolites, extracts, or concentrates.*

□ Yes (Goes to next question; supplement table)

□ No (Skips to next section)

Supplement	Used in past 12 months?	How often, on average, do you use the product?	Reason for use. (Choose From A – I) A. Medical Needs B. Medical Deficiency C. Health Maintenance/Prevent Nutritional Deficiency D. Increase or Maintain Muscle Mass E. Increase Endurance F. Increase Energy G. Improve Exercise Recovery H. Enhance Immune System/Prevent Illness I. Other Reason
Dietary/Medicinal			
Vitamin E	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Vitamin D	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Vitamin C	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Vitamin B12	□ Yes □ No □	□ Once per day □ Once per week □ Once per month	

B-Complex Vitamins	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Multivitamin/minerals	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Zinc	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Iodine	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Calcium	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Iron	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Omega-3fatty acids (DHA/EPA)	□ Yes □ No	□ Once per day □ Once per week □ Once per month	

Omega-3Fatty acids (ALA)	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Probiotics	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Muscle Building/Improved Energy			
Branched chain amino acids	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Creatine	□ Yes □ No	□ □ Once per day □ Once per week □ Once per month	
Caffeine	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Energy Drinks	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Performance Foods			
Sports drinks	□ Yes □ No	□ Once per day □ Once per week	

		□ Once per month	
Electrolytes	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Bicarbonate	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Carbohydrate sports bar	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Protein Powder	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Protein Bar	□ Yes □ No	□ Once per day □ Once per week □ Once per month	
Sports Gels	□ Yes □ No	□ Once per day □ Once per week □ Once per month	

If you currently use a product/supplement <u>not included</u> on this list please describe the product including the primary ingredient, how often you use it and reason for use.

Product_____

Supplement_____

Once per day □ Once per week □ Once per month □

Reason for Use

_____

_____

Product_____

Ingredient_____

Once per day □ Once per week □ Once per month □

Reason for Use

_____

_____

Rank the top 3 sources of information you rely on regarding nutrition

_____ Academic Journal

_____ Athletic Trainer/ Strength and Conditioning Coach

_____ Coach

_____ Dietitian

_____ Nutritionist

_____ Doctor

_____ Family/Friend

_____ Internet Search

_____ Mass Media (Magazine, Radio, TV)

_____ Social Media (Facebook, Twitter)

_____ Team Mates

□ I don't rely on any of the above

Appendix B. Supplemental Table 1: PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	

FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Appendix C. Example search strategy for systematic review.

((((((((((((((((((((((((((((((("immunoglobulins"[MeSH Terms]) OR Immunoglobulin*) OR IgE) OR IgD) OR IgM) OR IgA) OR IgG) OR Platelet[MeSH Terms]) OR Platelet*) OR Basophil[MeSH Terms]) OR Basophil*) OR Eosinophil[MeSH Terms]) OR Eosinophil*) OR "t lymphocyte subsets"[MeSH Terms]) OR t cell*) OR "b lymphocyte subsets"[MeSH Terms]) OR B cell*) OR Monocyte[MeSH Terms]) OR Monocyte*) OR Neutrophil[MeSH Terms]) OR Neutrophil*) OR Leukocyte[MeSH Terms]) OR Lymphocyte*) OR Lymphocyte*[MeSH Terms]) OR Leukocyte*) OR white blood cells) OR "white blood cells") OR white blood cell) OR "white blood cell") OR NK) OR "natural killer t cells"[MeSH Terms]) OR "natural killer cells") OR natural killer cells) OR natural killer cell) OR "natural killer cell") OR "immunity"[MeSH Terms]) OR immune)) OR (((tumor necrosis factor[MeSH Terms]) OR interleukin[MeSH Terms]) OR ((((((((((((((("fibrinogen"[MeSH Terms]) OR fibrinogen) OR TNF) OR "tumor necrosis factor alpha"[MeSH Terms]) OR tumour necrosis factor) OR "tumour necrosis factor") OR "tumor necrosis factor") OR IL-6) OR interleukin) OR "interleukin") OR CRP) OR "c reactive protein") OR C-Reactive Protein[MeSH Terms]) OR inflammat*) OR "inflammation"[MeSH Terms])))) AND (((((("plant based") OR "plant-based") OR vegan*) OR "vegans"[MeSH Terms]) OR *vegetarian) OR vegetarian*) OR vegetarian[MeSH Terms])

Appendix D. Modified Newcastle Ottawa Scale assessing of the quality of studies.

	Acosta-Navarro et al [423], Navarro et al. [89] [1]	Justification	Ambroszkiewicz et al. [424]	Justification	Chen et al. [392]	Justification	Chen et al. [391]	Justification	Chuang et al. [393]	Justification	Dong and Scott [88]	Justification
Sample selection criteria **Selection:** (Maximum 3 stars)												
1) Representativeness of the sample: a) Truly representative of the average in the target population. ★ (all subjects or random sampling) b) Somewhat representative of the average in the target population. ★ (non-random sampling) c) Selected group of users. d) No description of the sampling strategy.	1b ★	small sample size	1b ★	children aged 4.5-9	1b ★	All pts undergoing general health examination, but enrolled first come, first served	1b ★	females only	1b ★	large sample based on health records of pts in clinics, but not clear how vegetarian and non-vegetarian cases/controls were identified	1c	pts of a vegetarian society conference + very small non-veg group
2) Non-respondents: a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. ★ b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. c) No description of the response rate or the characteristics of the responders and the non-responders.	2a ★	-	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled
3) Ascertainment of the exposure (risk factor): a) Validated measurement tool. ★ b) Non-validated measurement tool, but the tool is available or described. c) No description of the measurement tool. NOTE - Study must say 'validated' to score star	3b	-	3b	tool described, but not clear if validated	3c	No description - general diet only	3c	No description of questionnaire used	3a ★	validated tool	3b	tool described, but not clear if validated

Comparability: (Maximum 2 stars) *1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.* a) The study controls for the most important factor (BMI). ★ b) The study controls for any additional factors. ★ (Smoking and physical activity) Note: for ★ on 1b - both PA and smoking needs to be controlled	1b ★	ANOVA analysis performed due to differences in PA	1a ★	PA not considered BMI similar between groups, other factors not discussed	1a ★	Sig. differences in smoking	1a ★ 1b ★	Did not statistically adjust, but exclusion criteria would have limited confounders somewhat	1b ★	Did not adjust for BMI (differed significantly between groups), but did adjust for age, sex, PA, alcohol and study site	-	Does not appear to adjust for confounders
Outcome: (Maximum 2 stars)												
1) Assessment of the outcome: a) Independent blind assessment. ★ b) Record linkage. ★ c) Self report. d) No description. *2) Statistical test:* a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). ★ b) The statistical test is not appropriate, not described or incomplete.	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2b	-
Total ★ (/7) [2]	5		4		4		5		5		1	

	Famodu et al. [394]	Justification	Fontana et al. [395]	Justification	Fontana et al. [396]	Justification	Franco-de-Moraes et al. [397]	Justification	Gorczyca et al. [398]	Justification	Haddad et al. [399]	Justification
Sample selection criteria **Selection:** (Maximum 3 stars)												

1) Representativeness of the sample: a) Truly representative of the average in the target population. ★ (all subjects or random sampling) b) Somewhat representative of the average in the target population. ★ (non-random sampling) c) Selected group of users. d) No description of the sampling strategy.	1d	no description of sampling strategy (states members of Adventist Seminary Institute of West Africa.) + non-vegetarians	1c	small select sample, not representative raw vegans	1c	small sample, not clear how controls recruited, not representative	1b ★	convenience sample	1c	parents of non-vegetarian children not randomly selected	1c	small sample, unlikely to be representative
2) Non-respondents: a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. ★ b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. c) No description of the response rate or the characteristics of the responders and the non-responders.	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled
3) Ascertainment of the exposure (risk factor): a) Validated measurement tool. ★ b) Non-validated measurement tool, but the tool is available or described. c) No description of the measurement tool. NOTE - Study must say 'validated' to score star	3b	tool described in supporting reference, but not described if validated	3b	WFR - but no mention of validating	3b	WFR but no mention of validation	3c	-	3b	FR used but unsure if validated	3b	FR (trained) but no mention of validation

Comparability: (Maximum 2 stars) *1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.* a) The study controls for the most important factor (BMI). ★ b) The study controls for any additional factors. ★ (Smoking and physical activity) Note: for ★ on 1b - both PA and smoking needs to be controlled	1a ★	did not adjust for confounders, although no difference in BMI. No description of smoking status or PA	-	Did not adjust for confounders (differences in BMI) smoking same between groups however no description of PA between Ve and NV	-	Did not adjust for confounders (differences in BMI) smoking same between groups however no description of PA between Ve and NV	Nil	BMI and PA not controlled	1a ★	did not adjust for confounders, or control for PA or smoking. Height and weight not sig different between groups	1b ★	BMI sig diff between groups. PA and Smoking no sig diff
Outcome: (Maximum 2 stars)												
1) Assessment of the outcome: a) Independent blind assessment. ★ b) Record linkage. ★ c) Self report. d) No description. *2) Statistical test:* a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). ★ b) The statistical test is not appropriate, not described or incomplete.	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-
Total ★ (/7)	3		2		2		3		3		3	

	Krajcovicova-Kudlackova et al. [400]	Justification	Malter et al. [94]	Justification	Mezzano et al. [401]	Justification	Montalcini et al. [402]	Justification	Ou et al. [403]	Justification	Paalani et al. [404]	Justification
Sample selection criteria **Selection: (Maximum 3 stars)**												

1) Representativeness of the sample: a) Truly representative of the average in the target population. ★ (all subjects or random sampling) b) Somewhat representative of the average in the target population. ★ (non-random sampling) c) Selected group of users. d) No description of the sampling strategy.	1b ★	random sampling but no description of strategy	1c	small sample, not clear how selected from Heidelberg study (veg) or research centre (non-veg)	1d	not described	1b ★	small sample, but recruited following newspaper ads	1c	chronic dialysis patients	1a ★	-
2) Non-respondents: a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. ★ b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. c) No description of the response rate or the characteristics of the responders and the non-responders.	2c	No description of those not enrolled	2c	No description of those not enrolled	2c	No description of those not enrolled	2c	No description of those not enrolled	2c	No description of those not enrolled	2a ★	-
3) Ascertainment of the exposure (risk factor): a) Validated measurement tool. ★ b) Non-validated measurement tool, but the tool is available or described. c) No description of the measurement tool. NOTE - Study must say 'validated' to score star	3c	tool not described	3c	no tool	3b	tool described, but not clear if validated	3b	tool described, but not clear if validated	3c	tool not described	3b	tool described, unclear if validated
Comparability: (Maximum 2 stars)												
1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled. *a) The study controls for the most important factor (BMI). ★* *b) The study controls for any additional factors. ★ (Smoking and physical activity)* *Note: for ★ on 1b - both PA and smoking needs to be controlled*	-	smoking controlled for, however BMI sig difference and no description of PA	-	did not adjust for confounders (differences in other risk factors between groups)	1a ★	matched by BMI, age, sex - no mention of PA	1a ★	matched by BMI, age, sex. PA sig different between groups	-	age and sex matched, but BMI still sig different between groups, and not adjusted	-	Baseline data not available

Outcome: (Maximum 2 stars) *1) Assessment of the outcome:* a) Independent blind assessment. ★ b) Record linkage. ★ c) Self report. d) No description. *2) Statistical test:* a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). ★ b) The statistical test is not appropriate, not described or incomplete.	1a ★ 2b	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-
Total ★ (/7)	2		2		3		4		2		4	

	Pinto et al. [425]	Justification	Pongstaporn et al. [86]	Justification	Refsum et al. [406]	Justification	Sebekova et al. [407]	Justification	Sebekova et al. [408]	Justification	Su et al. [409]	Justification
Sample selection criteria **Selection:** (Maximum 3 stars)												
1) Representativeness of the sample: a) Truly representative of the average in the target population. ★ (all subjects or random sampling) b) Somewhat representative of the average in the target population. ★ (non-random sampling) c) Selected group of users. d) No description of the sampling strategy.	1b ★	recruited via email, adverts and email circulation	1d	-	1b ★	large numbers but obtained from cardiac clinic	1d	-	1d	-	1c	small select sample
2) Non-respondents: a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. ★ b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. c) No description of the response rate or the characteristics of the responders and the non-responders.	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled
3) Ascertainment of the exposure (risk factor): *a) Validated measurement tool. ★* *b) Non-validated measurement tool, but the tool is available or described.* *c) No description of the measurement tool.* *NOTE - Study must say 'validated' to score star*	3a ★	validated FFQ	3c	tool not described	3b	tool described, but not clear if validated	3b	tool described, but not clear if validated	3b	tool described, but not clear if validated	3c	tool not described

Comparability: (Maximum 2 stars)												
1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled. *a) The study controls for the most important factor (BMI).* ★ *b) The study controls for any additional factors.* ★ *(Smoking and physical activity)* *Note: for* ★ *on 1b - both PA and smoking needs to be controlled*	1a ★	BMI controlled - Nil for PA	-	-	-	does not control for confounders, unclear if potential confounders differed between groups	-	BMI sig different. Smoking controlled but not PA	-	does not control for confounders	1a ★	BMI not sig diff

Outcome: (Maximum 2 stars) *1) Assessment of the outcome:* a) Independent blind assessment. ★ b) Record linkage. ★ c) Self report. d) No description. *2) Statistical test:* a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). ★ b) The statistical test is not appropriate, not described or incomplete.	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-
Total ★ **(/7)**	5		2		3		2		2		3	

	Suwannuruks et al. [410]	Justification	Szeto et al. [136]	Justification	Tungtrongchitr et al. [92]	Justification	Wu et al. [411]	Justification	Yang et al. [422]	Justification
Sample selection criteria **Selection:** (Maximum 3 stars)										
1) Representativeness of the sample: a) Truly representative of the average in the target population. ★ (all subjects or random sampling) b) Somewhat representative of the average in the target population. ★ (non-random sampling) c) Selected group of users. d) No description of the sampling strategy.	1c	small select sample	1c	small select sample	1c	small select sample, not clear how controls recruited	1b ★	patients on HD	1c	select sample, not representative

2) Non-respondents: a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. ★ b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory. c) No description of the response rate or the characteristics of the responders and the non-responders.	2c	no description of those not enrolled	2c	no description of those not enrolled	2c	no description of those not enrolled	2a ★	-	2c	no description of those not enrolled
3) Ascertainment of the exposure (risk factor): *a) Validated measurement tool. ★* *b) Non-validated measurement tool, but the tool is available or described.* *c) No description of the measurement tool.* *NOTE - Study must say 'validated' to score star*	3c	tool not described	3c	tool not described	3c	tool not described	3b	tool described, but not clear if validated	3b	tool described, but not clear if validated

Comparability: (Maximum 2 stars) *1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.* *a) The study controls for the most important factor (BMI). ★* *b) The study controls for any additional factors. ★ (Smoking and physical activity)* *Note: for ★ on 1b - both PA and smoking needs to be controlled*	-	not adjusted for confounders	-	-	-	-	-	some differences between groups, did not adjust	1a ★ 2a ★	Table 1 footnotes suggest adjusted for covariates
Outcome: (Maximum 2 stars)										

1) Assessment of the outcome: a) Independent blind assessment. ★ b) Record linkage. ★ c) Self report. d) No description. *2) Statistical test:* a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). ★ b) The statistical test is not appropriate, not described or incomplete.	1a ★ 2b	stats test not described	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-	1a ★ 2a ★	-
★Total = /7	1		2		2		4		4	

BMI, body mass index; diff, difference; FFQ, food frequency questionnaire; FR, food record; HD, hemodialysis; NV, Non-vegetarian; PA, physical activity; Pts, patients; sig, significant; ve, vegan; veg, vegetarian; WFR, weighted food record.

[1] Two separate papers identified reporting on same study participants, with different outcome marker/s

[2] Studies assessed using the modified Newcastle Ottawa Scale can achieve 7 stars in total. Studies attracting 7 stars are of high quality while studies attracting 0 stars are of low quality. The criteria in the first column explains the criteria to attain a star.

★, Sample selection criteria met

Appendix E. GRADE assessment of the quality of the body of evidence in observational studies for each outcome.

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Vegetarian-based**	**control**	**Relative (95% CI)**	**Absolute (95% CI)**		
CRP												
19	Observational studies	serious [a]	serious [b]	not serious	not serious	None [3]	1844	4736	-	MD **0.62 lower** (0.93 lower to 0.30 lower)	⊕◯◯◯ VERY LOW	IMPORTANT
Fibrinogen												
3	Observational studies	serious [1]	not serious [4]	not serious	serious	none [3]	112	96	-	MD **0.22 lower** (0.41 lower to 0.04 lower)	⊕◯◯◯ VERY LOW	IMPORTANT
Thrombocytes												
7	Observational studies	serious [1]	not serious [4]	not serious	not serious	none	663	507	-	MD **8.24 higher** (3.35 lower to 19.82 higher)	⊕◯◯◯ VERY LOW	IMPORTANT
Leukocytes												

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Vegetarian-based**	**control**	**Relative (95% CI)**	**Absolute (95% CI)**		
11	Observational studies	serious [1]	serious [2]	not serious [3]	not serious	none	944	970	-	MD **0.62 lower** (1.13 lower to 0.10 lower)	⊕◯◯◯ VERY LOW	IMPORTANT

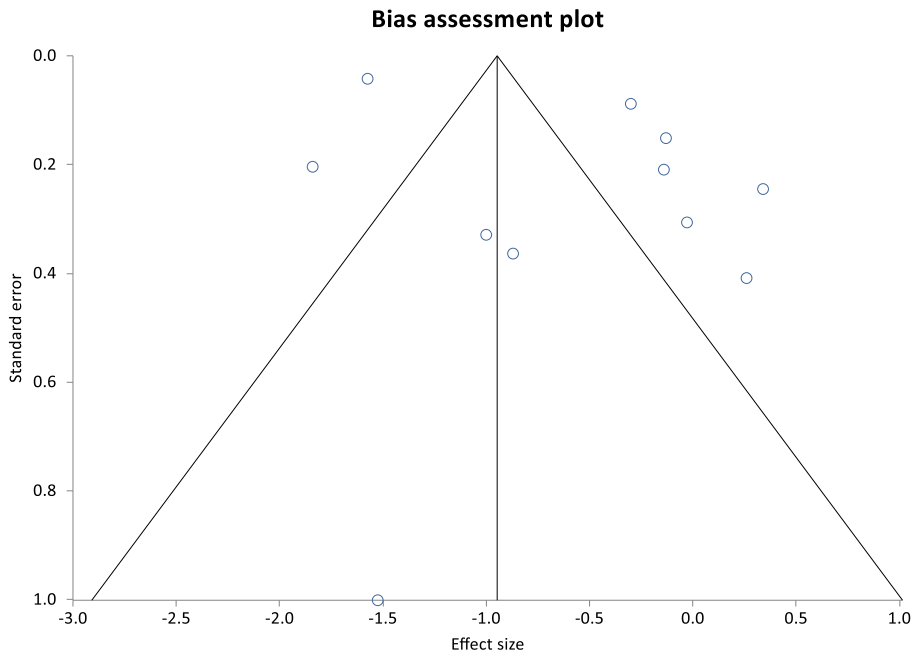
CI, Confidence interval; MD, Mean difference

[1] The studies were viewed as being in the category of 'serious limitation'. This category was selected as the risk of bias assessments for each study using a modified Newcastle Ottawa Scale resulted in many studies scoring poorly (majority 4 or less /7). In accordance with the GRADE guidelines, 'high risk' needed to be categorized as either 'serious limitations' or 'very serious limitations'. In view of the potential implications of the 'high risk' aspects on the quality of the body of evidence, 'serious limitations' was selected.

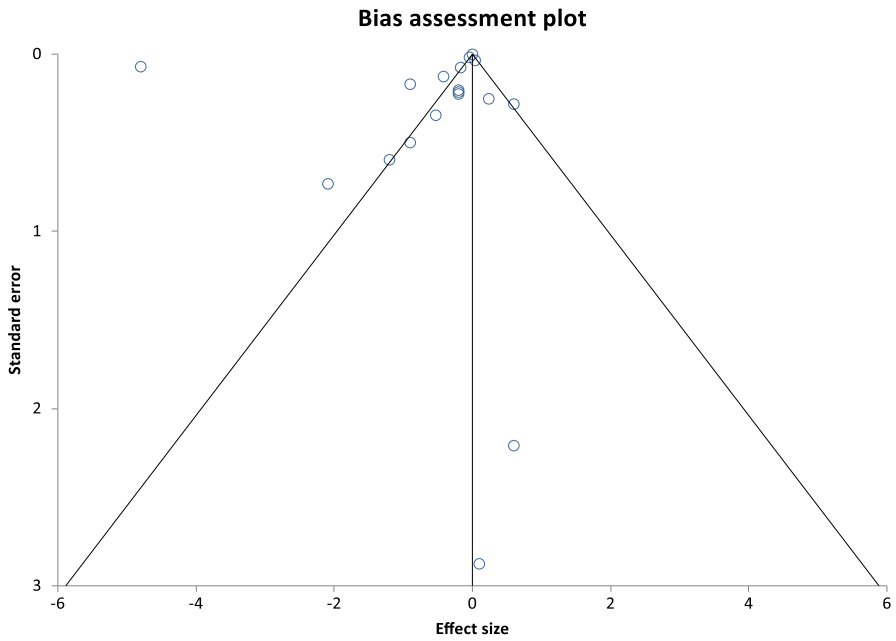
[2] I squared value of 100%, indicating considerable heterogeneity

[3] Funnel plot does not indicate publication bias

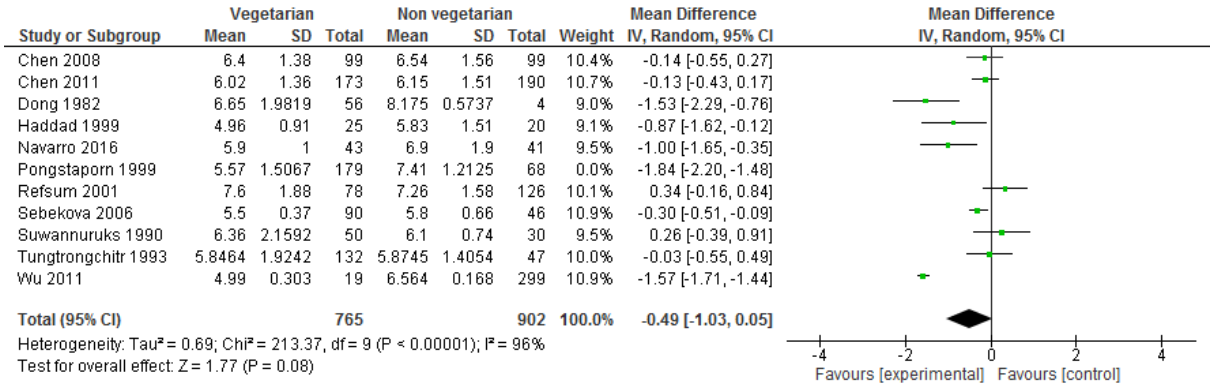
[4] I squared value of <50% indicating minimal heterogeneity



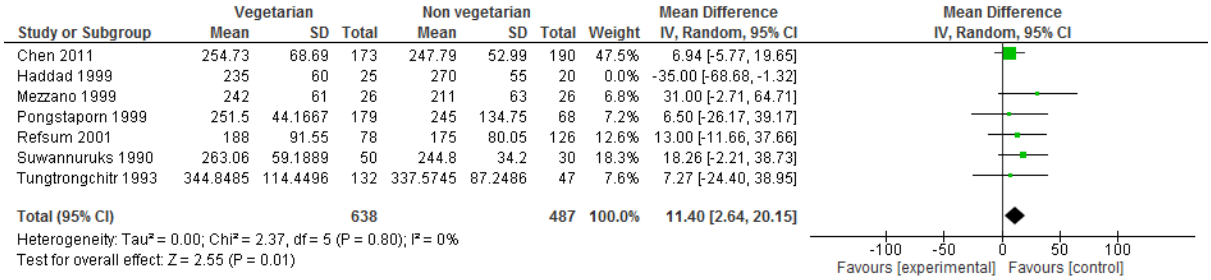
Appendix F. Bias assessment plot for leukocyte concentration with Egger's test applied. Egger bias 4.439487; 95% CI: -0.439381, 9.318356; P = 0.0697.



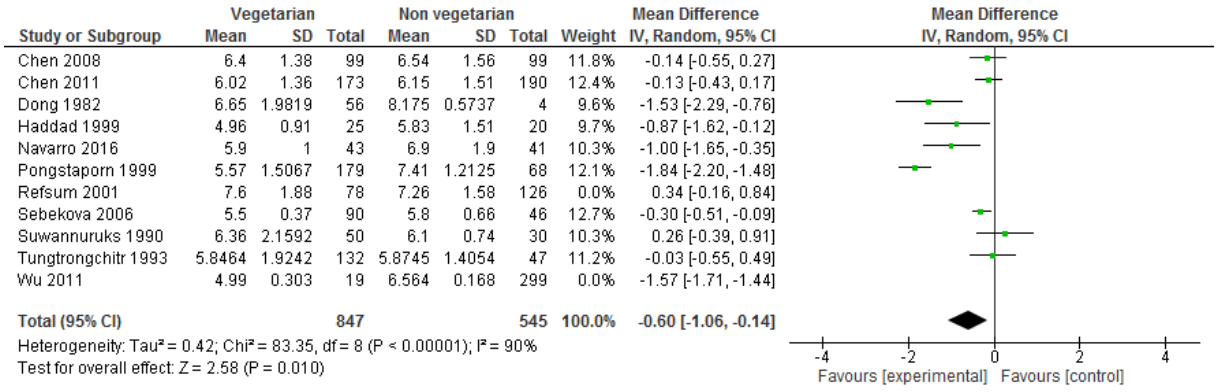
Appendix G. Bias assessment plot for CRP concentration with Egger's test applied. Egger bias: -5.165008; 95% CI: -13.583609, 3.253593; P = 0.2118



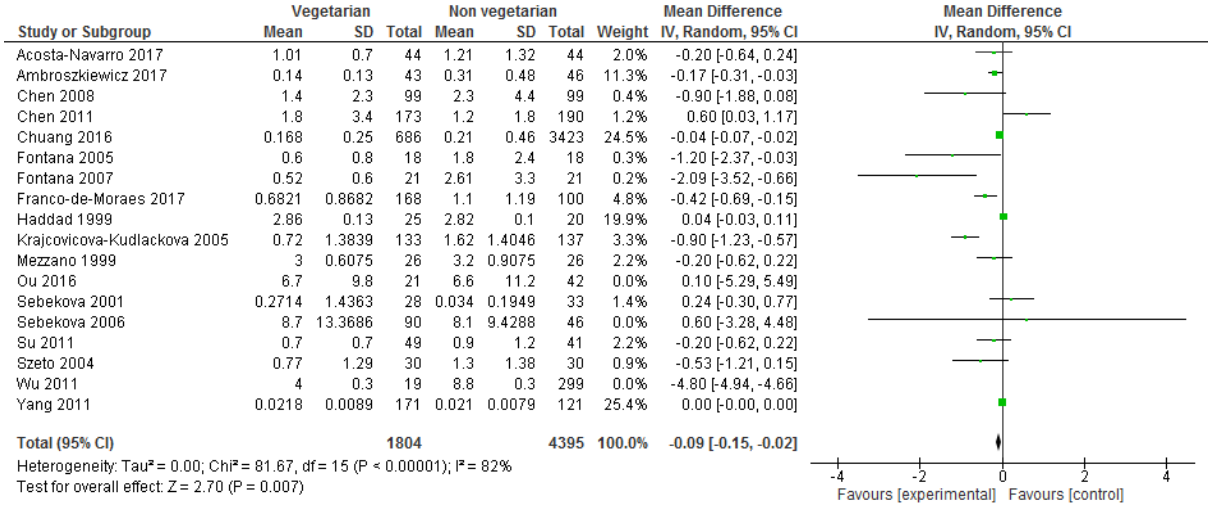
Appendix H. Sensitivity analysis for leukocyte (103/µL) values between those following vegetarian-based dietary patterns and non-vegetarian dietary patterns (cross-sectional studies) with Pongstaporn et al omitted. Diamond indicates weighted mean difference with 95% confidence intervals.



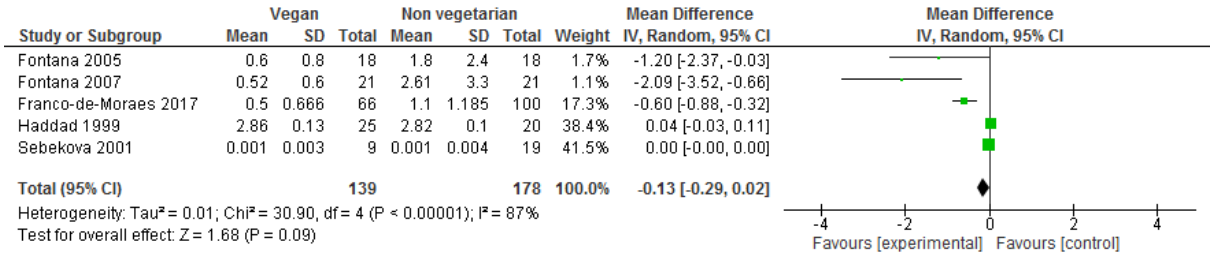
Appendix I. Sensitivity analysis for thrombocyte (x109/L) counts between those following vegetarian-based dietary patterns and non-vegetarian dietary patterns (cross-sectional studies) with Haddad et al omitted. Diamond indicates weighted mean difference with 95% confidence intervals.



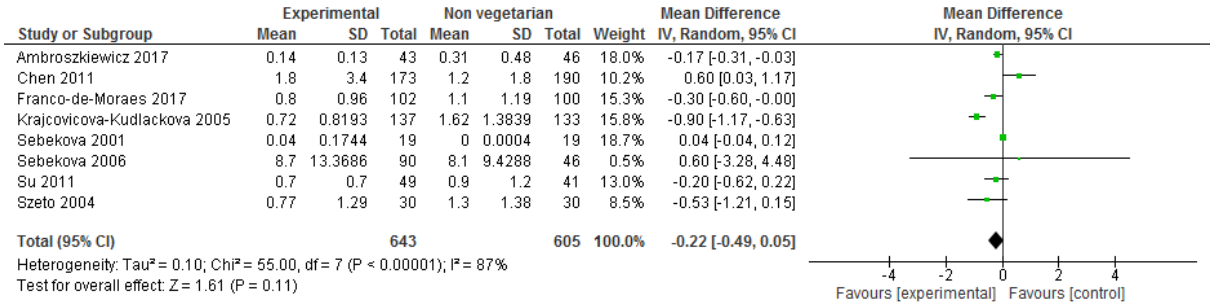
Appendix J. Sensitivity analysis for leukocytes (103/µL) between those following vegetarian-based and non-vegetarian based dietary patterns (cross-sectional studies) with studies omitted where participants were receiving haemodialysis treatment, CVD and/or T2DM were omitted. Diamond indicates weighted mean difference with 95% confidence intervals.



Appendix K. Sensitivity analysis for CRP (mg/L) between those following vegetarian-based and non-vegetarian based dietary patterns (cross-sectional studies) with studies omitted where participants were receiving haemodialysis treatment, CVD and/or T2DM were omitted. Diamond indicates weighted mean difference with 95% confidence intervals



Appendix L. Difference in CRP (mg/L) values between those following vegan dietary patterns and non-vegetarian dietary patterns (cross-sectional studies). Diamond indicates weighted mean difference with 95% confidence intervals



Appendix M. Difference in CRP (mg/L) values between those following Lacto-ovo-vegetarian dietary patterns and non-vegetarian dietary patterns (cross-sectional studies). Diamond indicates weighted mean difference with 95% confidence interval

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Vegetarian Diet**	**Mixed non-vegetarian diet**	**Relative (95% CI)**	**Absolute (95% CI)**		
CRP												
4	randomized trials	serious [1]	serious [2]	serious [3]	serious [4]	nil	114	116	-	MD 1.07 **lower** (2.75 lower to 0.61 higher)	⊕◯◯◯ VERY LOW	IMPORTANT

MD – mean difference,

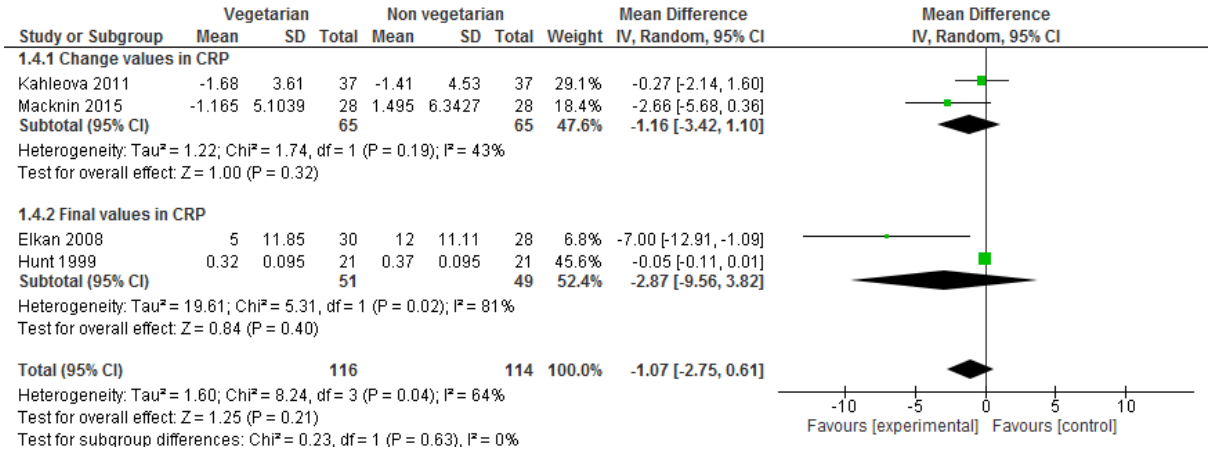
[1] The studies were viewed as bring in the category of 'serious'. This category was selected as despite risk of bias assessments for each study mainly compromising of 'low risk' and 'unclear risk' (see risk of bias assessment charts) the 'other bias's domain had 100% of studies in the 'high risk category'. In accordance with the GRADE guidelines, 'high risk' should be downgraded by one level when "one criterion or some limitations for multiple criteria, sufficient to lower confidence in the estimate of effect" was selected.

[2] Inconsistency was deemed to be 'not serious' as the I squared value of 53%, which only slightly exceeded the range (50%-75%) which "likely indicates substantial heterogeneity" as outlined in the Cochrane handbook.

[3] The studies were viewed as bring in the category of 'serious'. This category was selected, as there was considerable inconsistency between the populations regarding the main review question. For example, Elkan et als, 2008 study examined participants with rheumatoid arthritis, Kahleova et al, had patients had T2DM and Macknin et al, 2015 had participants who were children with a BMI > 95th % for age/sex + cholesterol >169mg/dL.

[4] 95% CI does not include an effect, 95% CI does not include appreciable benefit or harm, however less than 400 participants available, therefore the decision was made to downgrade the quality of evidence.

Appendix N. GRADE assessment of the quality of the body of evidence – CRP intervention studies.



Appendix O. Change in C-reactive protein (mg/L) between vegetarian dietary patterns and non-vegetarian control dietary patterns (presented as sub-groups based on mean final or change values for readability). Diamond indicates weighted mean difference with 95% confidence intervals.

Appendix P. Cochrane risk of bias assessment of interventional studies.

Elkan et al. (72)		
Bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (selection bias)	Unclear risk	"Participants were randomly assigned using a minimization technique" - no specific detail on how this was performed.
Allocation concealment (selection bias)	Unclear risk	Not Specified
Blinding of participants and researchers (performance bias)	High risk	Participants aware of dietary group after first check-up (3 months into 1-year trial) - No description of blinding by researchers.
Blinding of outcome assessment (detection bias)	Low risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	High Risk	Dropout rate >25% in vegan group after 1 year. Intention-to-treat (ITT) not used
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	High Risk	CRP appears significantly higher in control group at baseline.
Kahleova et al. (73)		
Bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomized, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and researchers (performance bias)	Unclear risk	Not possible to blind personnel, unclear if patients blinded
Blinding of outcome assessment (detection bias)	Low risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	Low risk	16% drop out, but similar between groups and ITT used
Selective reporting (reporting bias)	Unclear risk	Protocol available, but insufficient information to determine if all outcomes reported
Other bias	High risk	Smoking higher in Control group at baseline
Hunt & Roughead. (81)		
Bias	**Authors' judgment**	**Support for judgment**

Random sequence generation (selection bias)	Unclear risk	Stated to be randomized, no details of randomisation method given
Allocation concealment (selection bias)	Unclear Risk	Not stated
Blinding of participants and researchers (performance bias)	High Risk	Not possible to blind researchers. Not possible to blind participants (cross-over) which may have affected performance in different arms
Blinding of outcome assessment (detection bias)	Low risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	Low risk	Nil drop out
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	High Risk	Nil washout period

Kjeldsen-Kragh et al. (74, 75) [1]

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Unclear Risk	Stated to be randomized, no details of randomisation method given
Allocation concealment (selection bias)	Unclear Risk	Not stated
Blinding of participants and researchers (performance bias)	High risk	Single blind trial - Participants aware of dietary group after first check-up (3 months into 1-year trial).
Blinding of outcome assessment (detection bias)	Low risk	Clinicians/GP's blinded + outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	High risk	30% drop out (even though ITT used and similar between groups)
Selective reporting (reporting bias)	Unclear Risk	The study protocol is not available
Other bias	High Risk	Insufficient baseline data reported to determine differences between groups + substantial difference in kJ intake between interventions and control

Macknin et al. (76)

Bias	Authors' judgment	Support for judgment
Random sequence generation (selection bias)	Low Risk	Randomized using an SAS computer program 1:1 in blocks of 4 families stratified by the child's age group (age strata 9-13 years vs 14-18 years)

Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and researchers (performance bias)	Unclear risk	Not stated
Blinding of outcome assessment (detection bias)	Low risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	High Risk	>10% drop out, both in intervention group, no ITT
Selective reporting (reporting bias)	Low Risk	The study protocol is available and all pre-specified outcomes of interest to the review have been reported in the pre specified way
Other bias	High Risk	Baseline CRP and IL-6 does not appear to be similar.

Nenonen et al. (77)

Bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (selection bias)	Unclear risk	Stated to be randomized, no details of randomisation method given
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding of participants and researchers (performance bias)	Unclear risk	Not stated
Blinding of outcome assessment (detection bias)	Low risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	High risk	higher drop out in intervention, related to intervention
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Baseline CRP between groups unclear

Richter et al (78)

Bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (selection bias)	Unclear Risk	Not stated
Allocation concealment (selection bias)	Unclear Risk	Not stated
Blinding of participants and researchers (performance bias)	High risk	Would not be possible to blind participants or personnel as food was provided. Whilst this may not have affected measures, it may have affected participant behaviour during intervention and control periods

Blinding of outcome assessment (detection bias)	Low risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	Low risk	No missing outcome data
Selective reporting (reporting bias)	Unclear Risk	The study protocol not available
Other bias	Low risk	4-week washout period,

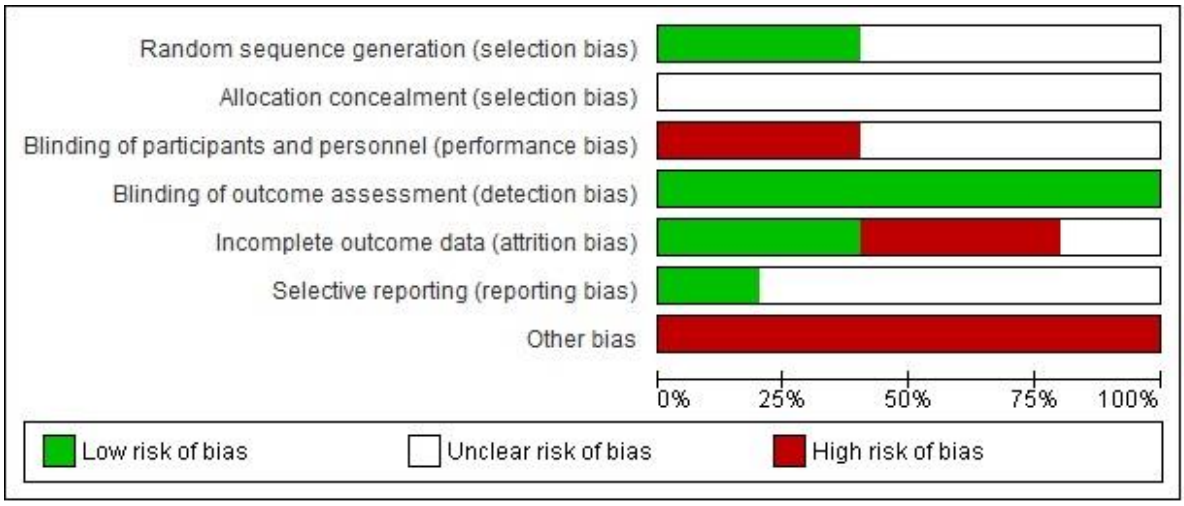
Sköldstam et al. (80)		
Bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (selection bias)	Unclear Risk	Not stated
Allocation concealment (selection bias)	Unclear Risk	Not stated
Blinding of participants and researchers (performance bias)	Unclear Risk	Not stated
Blinding of outcome assessment (detection bias)	Low Risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	Low risk	<5% drop out rate
Selective reporting (reporting bias)	Unclear risk	Protocol not described
Other bias	High Risk	Some bio-markers not comparable at baseline

Sköldstam (79)		
Bias	**Authors' judgment**	**Support for judgment**
Random sequence generation (selection bias)	Unclear	Not stated
Allocation concealment (selection bias)	Unclear Risk	Not stated
Blinding of participants and researchers (performance bias)	high risk	Not possible to blind researchers. Not possible to blind participants (pre-post) which may have affected performance in different arms
Blinding of outcome assessment (detection bias)	Low Risk	Not stated - although outcomes unlikely to be influenced by blinding (blood bio-markers)
Incomplete outcome data (attrition bias)	unclear risk	<10%, but unclear at which time pts dropped out
Selective reporting (reporting bias)	Unclear Risk	Protocol not described

Other bias	Unclear Risk	Base line data not reported

CRP, C-Reactive Protein; ITT, intention to treat; SAS, Statistical Analysis System.

[1] Kjeldsen-Kragh et al 1995 and 1991 - same participants/study, different outcomes reported



Appendix Q. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

Appendix R. Participant flowchart and communication - Exercise trial.