# Dietary intake and food sources of omega-3 polyunsaturated fatty acids of vegans living in New Zealand

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**Feiran (Fiona) Li**

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# Abstract

**Background:** The vegan diet, which excludes all animal products, poses a high risk of inadequate intake of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs). N-3 LCPUFAs are essential for cardiovascular health, cognitive function, and inflammatory regulation. There is currently limited evidence on the dietary intakes and food sources of n-3 LCPUFAs in vegans.

**Objective:** To investigate the intake, adequacy and main food sources of n-3 LCPUFAs in New Zealand vegans.

**Methods:** This study used a four-day food record to assess the dietary intake of 212 (155 female) New Zealand vegans including energy, total fat, polyunsaturated fatty acids (PUFAs), and omega-3 and omega-6 fatty acid intake. The study determined the adequacy of linoleic acid (LA), alpha-linolenic acid (ALA), and n-3 LCPUFAs by comparing intakes with the New Zealand National Health and Medical Research Council (NHMRC) recommended intakes and calculated the proportion of each fatty acid in the total intake. The main food sources of n-3 LCPUFA were also determined.

**Results:** Mean energy, total fat, polyunsaturated fatty acid, and LA intakes were significantly higher in males than in females, whereas there were no significant differences in intakes of n-3 LCPUFAs, ALA, EPA, DPA, and DHA. Most participants had adequate intakes of LA and ALA, but only a few women met the Adequate Intake (AI) for n-3 LCPUFA. The main food sources were nuts and seeds, mixed dishes and grains, while seaweed dominated EPA and DHA intake.

**Conclusion:** The majority of the participants in this study had inadequate n-3 long-chain polyunsaturated fatty acid intakes. It may be necessary to optimise the dietary intake of n- 3 long-chain fatty acids encouraging the consumption of foods rich in these nutrients.



**Key words:** omega-3, omega-6, LCPUFA, vegan, food sources

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# Abbreviations

| | |
|---|---|
| AI | Adequate Intake |
| ALA | Alpha-linolenic Acid, 18:3n-3 |
| ARA | Arachidonic Acid, 20:4n-6 |
| BMI | Body Mass Index |
| DHA | Docosahexaenoic Acid, 22:6n-3 |
| DPA | Docosapentaenoic Acid, 22:5n-3 |
| DXA | Dual-energy X-ray Absorptiometry |
| EAR | Estimated Average Requirement |
| EPA | Eicosapentaenoic Acid |
| FFQ | Food Frequency Questionnaire |
| LA | Linoleic Acid, 18:2n-6 |
| LCPUFA | Long Chain Polyunsaturated Fatty Acid |
| mg | Milligrams |
| MJ | Mega-joule |
| n-3 | Omega 3 |
| n-6 | Omega 6 |
| NHMRC | National Health and Medical Research Council |
| PUFA | Polyunsaturated Fatty Acids |
| UL | Tolerable Upper Intake Level |

# Chapter 1: Background and Purpose of the Study

## 1.1 Background

The vegan diet eliminates all animal products including meat, dairy, fish and eggs from consumption. People following a vegan diet rely on plant-based foods to meet their dietary requirements (Orlich et al., 2014; Papier et al., 2019). In recent years, the number of people choosing to follow a vegan diet has significantly increased worldwide. People choose to adhere to a vegan diet for ethical, environmental and health-related reasons (Janssen et al., 2016; Leitzmann, 2014; Lemale et al., 2019). There are almost 1% of New Zealanders who adhere to a vegan diet (Milfont et al., 2021).

One motivation for following a vegan diet is health. However, vegan diets may lead to a number of nutritional deficiencies including iron, calcium, B12, high quality protein as well as omega-3 long-chain polyunsaturated fatty acids (n-3LCPUFAs) due to the exclusion of animal products (Craig, 2009). These deficiencies may be particularly concerning for vulnerable populations such as pregnant and childbearing age women who have an increased need for docosahexaenoic acid (DHA) to support fetal development and brain health (Swanson et al., 2012; Coletta et al., 2010). These specific vegan populations may be at a higher risk of DHA deficiency and require careful monitoring of their n-3LCPUFA intake.

The omega-3 long chain polyunsaturated fatty acids (n-3LCPUFAs) are mainly present in fatty fish and some specified plant-based foods. The essential fatty acids alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and DHA is important for human health. They play a critical role in maintaining optimal nutrient balance and mitigating a variety of chronic diseases (Das, 2006; Husted & Bouzinova, 2016; Tocher et al., 2019).

Salmon, mackerel, sardines and other deep-sea fatty fish are the main food sources of n-3LCPUFAs for omnivores. However, vegans' food sources of n-3 LCPUFAs are limited. They include plant-based foods such as flaxseed, chia seed and hemp seeds. Although plant-based foods are rich in ALA, they lack EPA and DHA. ALA can be converted to EPA and DHA in the human body, but the efficiency of this conversion is limited and can be affected by factors such as age, sex and omega-6 intake (Wijendran & Hayes, 2004).

There is an intricate interaction between omega-3 and omega-6 fatty acids. Both groups of fatty acids are important for human health. Vegetable oils, seeds and nuts are a good source of omega-6 fatty acids. Omega-6 fatty acids compete with and regulate omega-3 fatty acids due to the sharing of metabolic enzymes between LA and ALA (Astorg et al., 2004; Blasbalg et al., 2011; Whelan & Fritsche, 2013). Characterised by an overabundance of omega-6 fatty

acids, the modern Western diet yields dietary n-6/n-3 ratios surpassing 20:1 (Simopoulos, 2002, 2016). These imbalanced n-6/n-3 ratios underscore the requirement for dietary management as the equilibrium of n-6 and n-3 LCPUFAs is pivotal for overall health. The conventionally accepted ratio of n-6 to n-3 polyunsaturated fatty acids approximates 4:1.

However, current dietary trends, typified by heightened consumption of processed foods, have created skewed ratios of 16:1 or higher, further limiting the conversion of ALA to DHA and EPA (Simopoulos, 2002, 2010, 2016).

Deficiencies in omega-3 fatty acids can give rise to a spectrum of health concerns, including cardiovascular complications, cognitive debilitation, and affective disorders (Liao et al., 2019; Mallick et al., 2021; Tocher et al., 2019). Vegans are at a higher risk of n-3LCPUFA deficiency and an imbalance in the n-6/n-3 ratio than those individuals who consume animal products (Kornsteiner et al., 2008; Mann et al., 2006; Pinto et al., 2017a; Sanders et al., 1978). Craddock et al. (2022) found that vegans have a significantly higher n-6/n-3 ratio of 9.11 (8.60-9.86) compared to omnivores, whose ratio is 6.23 (5.78-8.42), further underscoring the potential deficiency of n-3LCPUFAs in vegans. People who adhere to a vegan diet may need to monitor their omega-3 intake or incorporate specific plant-based sources of supplementation. It is recommended that males aged 19 years and over consume at least 1.3 g of ALA and 160 mg or more of total LC n-3 (DHA+EPA+DPA) (National Health and Medical Research Council,2006). Females are recommended to consume 0.8 g of ALA and more than 90 mg of total LC n-3 (DHA+EPA+DPA) per day.

Despite the concerns about n-3LCPUFA status in people following a vegan diet there is limited research on the dietary intake of n-3 LCPUFAs and the available food sources for vegans, particularly within New Zealand. Therefore, the aim of this study was to explore the dietary intake status and sources of n-3LCPUFAs among vegans in New Zealand. Due to the increasing prevalence of individuals choosing to adhere to a vegan diet and the promotion of plant-based foods within dietary guidelines the findings from this study will be of interest to individuals and health professionals.

## 1.2 Purpose of the Study

Inadequate dietary intake of n-3LCPUFAs and restricted food sources are issues for individuals following a vegan diet. Current evidence indicates that vegans may be at higher risk of omega-3 LCPUFA deficiency than other population groups who are not reliant on plant-based foods (Kornsteiner et al., 2008; Mann et al., 2006a; Pinto et al., 2017a; Sanders et al., 1978). There is currently limited evidence on vegan diets in New Zealand and the most recent national dietary survey which had limited data on vegan diets was published in 2008. Due to the growing popularity of the vegan diet, it is timely to assess the dietary status and food sources of omega-3 LCPUFAs.

## 1.3 Aim

To assess the dietary intake of omega-3 fatty acids and food sources among vegans living in New Zealand

## 1.4 Objectives

i) To determine the dietary intake of omega-3 fatty acids among vegans living in New Zealand.

ii) To assess the proportion of omega-6LCPUFAs and omega-3LCPUFAs in the diets of New Zealand vegans.

iii) To compare dietary intake of omega-3LCPUFAs among New Zealand vegans with recommended intakes

iv) To identify the food sources of omega-3LCPUFAs in the New Zealand vegans' diet.

## 1.5 Thesis Structure

This thesis consists of four main chapters with accompanying references and appendices. The first chapter is the introduction, which outlines the background and purpose for this study on the intake of n-3 LCPUFA and its food sources in people following a vegan diet. This chapter also describes the aims and objectives of the study and the contributions of the research team to the thesis.

Chapter 2 reviews the current literature on n-3 LCPUFA including their main functions, competing relationships, food sources, ideal intake ratios, and studies on the health effects of high n-6/n-3 ratios. This chapter also reviews the current evidence on the intake status of n-3 LCPUFA in people following a vegan diet. Relevant aspects related to dietary intake recommendations and supplement selection for vegan diets are also discussed.

Chapter 3 is the research manuscript presenting the methodology, results, discussion, and conclusion sections of the study. Detailed information on the recruitment methods used in this study, sample size, information on the study population, research methods and statistical analyses are provided here. In the results section the characteristics of the study population, n-3 and n-6 dietary intake of the participants are presented. Finally, the researchers compared them with the recommendations for specific nutritional reference values (NRVs). In addition, this chapter describes the main food sources of n-6 and n-3 PUFAs among the participants.

The final chapter summarises the main findings of this study, the strengths and limitations of the study, the implications of the findings and makes recommendations for further research.

## 1.6 Researchers' Contributions

| Researcher | Contributions |
|---|---|
| Fiona Li | Main researcher and author of this thesis. Involved in hosting participant visits, four days of food record data entry. Completed statistical analysis and interpretation of individual study data and presented the main findings. |
| Professor Pamela von Hurst | Main Supervisor and Vegan Study Principal Investigator Provided invaluable guidance and expertise throughout the research process. Helped design research protocols, assisted with data interpretation, and reviewed the thesis. |
| Professor Cathryn Conlon | Co-supervisor. Played a pivotal role in the refinement of the thesis, providing critical feedback and insights. |
| Karen Mumme | Provided essential assistance in data processing and conducted statistical analyses. |
| Hajar Mazahery | Vegan Study Manager. Contributed to recruiting and screening participants. |
| Rebecca Paul | Contributed to participant recruitment and data Entry. Assisted with 4DFR data entry and reviewed the data. |
| Owen Mugridge | Developed Research Web Pages and Assisted in the Creation of Online Questionnaires. |
| Lucie L'Estrange Hill, Abril Clark, Amelia Dunnett, Rebecca Pearce, Catherine Alice, Chelsea Corkindale | Fellow MSc students and members of the Vegan Nutrition Study Group. Involved in recruiting participants, data collection, 4DFR data entry into the vegan diet and editing the FoodWorks database. |

# Chapter 2: Review of Research Literature

## 2.1 Introduction

In recent years, vegan diets that depend exclusively on plant-based food sources have become popular in Western countries (Bakaloudi et al., 2021). Although vegans are still a relatively small proportion of the total population, the number of vegans worldwide has increased significantly over the past decade (Janssen et al., 2016; Leitzmann, 2014; Lemale et al., 2019). The 2018 New Zealand Attitudes and Values Study had 1.1 per cent of participants adhered to a vegan diet in a longitudinal study of 47,000 New Zealand participants (Milfont et al., 2021).

A vegan diet includes only plant-based foods and no animal products or foods with animal ingredients including honey, whey, casein, gelatin and any animal-derived supplements (Orlich et al., 2015; Papier et al., 2019). Plant-based food sources of fatty acids differ from animal- derived food sources. Therefore, a vegan diet may lead to a lower intake of n-3 LCPUFAs and PUFA imbalances that can affect health status. The omnivorous diet is more diverse as dietary fatty acid sources, including both plant-based and animal-based foods. The main plant-based food sources of fatty acids are nuts, seeds, avocados, vegetable oils (e.g., olive oil), and plant-based spreads. Vegetable oils are a rich source of n-6 fatty acids (Arachidonic acid - ARA, Linoleic acid - LA). However, since vegans do not eat fish, they may have lower intake of some long-chain omega-3 fatty acids (Eicosapentaenoic acid – EPA and Docosahexaenoic acid - DHA) which primarily are obtained from fatty fish.

Fatty acids are important components of cell membrane structure. Depending on the degree of saturation of the hydrocarbon chain, they are classified as saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) (Das, 2006; Husted & Bouzinova, 2016; Tocher et al., 2019). Alpha-linolenic acid (ALA) is an omega-3 fatty acid and linolenic acid (LA) is an omega- 6 fatty acid, both are essential and must be obtained from food. (Tocher et al., 2019). These fatty acids are important for brain function and cell growth in the human body. They are essential for maintaining the structural integrity and fluidity of cell membranes. In addition, they act as precursors for the synthesis of biologically active lipid mediators that regulate inflammatory and immune responses. N-3 PUFAs and their metabolites regulate anti-inflammatory effects and are essential in growth, development, and health maintenance (Liao et al., 2019; Mallick et al., 2021; Tocher et al., 2019).

Maintaining a proper balance between n-3 and n-6 fatty acids is essential for overall health (Simopoulos, 2002). Vegans typically obtain n-3 LCPUFAs from ALA found in plants like flaxseed, chia seeds, and hemp seeds, avoiding deep-sea fish and fish oil, which are the

primary sources of EPA and DHA. While food intake primarily determines tissue and blood concentrations of EPA and DHA, the body can also synthesise these fatty acids, albeit inefficiently. The conversion of ALA to EPA and DHA involves elongases and desaturases, enzymes that are limited in supply (Gerster, 1998). Both n-3 and n-6 fatty acids require the same enzymes for carbon chain extension, reducing the efficiency of ALA conversion (Gerster, 1998; Sanders, 2000). Higher LA intake in vegans' further limits ALA conversion (Gerster, 1998; Sanders, 2000). In addition, the imbalance between n-6 and n-3 ratios is associated with an increased risk of cardiovascular, brain and metabolic diseases and others (Dighriri et al., 2022; Schverer et al., 2020; Simopoulos, 2008; Berquin et al., 2008; Reddy & Sugie, 1988).

## 2.2 Overview of N-3 and N-6 Polyunsaturated Fatty Acids

Dietary fat is broken down into fatty acids after consumption, with concentrations in the blood and tissues associated with health. Fatty acids are classified into saturated and unsaturated fatty acids based on the degree of saturation of the hydrocarbon chain. Unsaturated fatty acids are classified into monounsaturated and polyunsaturated fatty acids by their structural characteristics (Das, 2006; Saunders et al., 2013; Tocher et al., 2019). Polyunsaturated fatty acids have more than one double bond and have between 18 and 22 carbon atoms. They have methyl groups (CH3) and carboxylic groups (COOH) connected at the two ends of the carbon chain (Das, 2006). Polyunsaturated fatty acids are widely present in two common forms of the n-3 and n-6 groups (Saunders et al., 2013). Scientists identify and differentiate these two families of PUFA by determining the position of the first double bond in the carbon chain of the PUFA molecule (Das, 2006; Tocher et al., 2019). The double bond found in the third carbon atom at the end of the methyl group of the PUFA molecules gives it its classification as omega

3. A polyunsaturated fatty acid with a C-C double bond on the sixth carbon atom is called omega-6 (Husted & Bouzinova, 2016). The number of carbon atoms and double bonds in a PUFA molecule determine the exact name of PUFA (Das, 2006b; Husted & Bouzinova, 2016; Saunders et al., 2013).

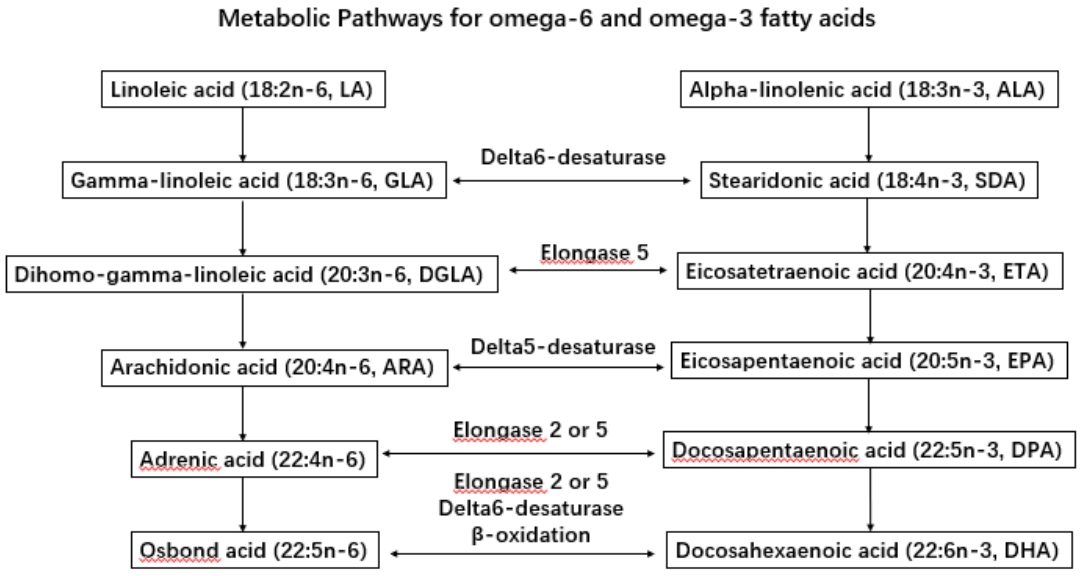
ALA and LA are parts of two families of fatty acids and both of them are essential fatty acids (EFAs) in human health (Saunders et al., 2013). These two essential fatty acids couldn't be synthesised by human selves. It means people need to consume adequate levels of ALA and LA in their diets to meet their nutritional needs (Burns-Whitmore et al., 2019). These two families of fatty acids have important functional physiological roles as basic nutrients. N-3 fatty acids have positive effects in a number of areas, including prevention of cardiovascular disease and immune function, regulation of body fat, reduction of inflammation, and

treatment of psychiatric disorders (de Lorgeril et al., 1994; Saunders et al., 2013; Simopoulos, 2002, 2010). LA and ALA are the parents of these two families of fatty acids (Saunders, 2013). Arachidonic acid (ARA), Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA) are highly unsaturated fatty acids because they have over 20 carbon atoms and more than three double bonds. They play critical physiological roles in promoting growth, development, and reproduction (Lands, 2014; Lands & Lamoreaux, 2012).

## 2.3 Competitive Relationship Between n-3 and n-6

The intake of fatty acids in the diet plays a crucial role in determining the status of polyunsaturated fatty acids in tissues and blood. It is essential to balance and appropriately consume n-3 LCPUFAs and n-6 LCPUFAs, as these two fatty acids share and compete for the same desaturase enzymes during breakdown and absorption processes (Lands, 2014; Lands & Lamoreaux, 2012; Saunders et al., 2013) (Figure 2.1).

**Figure 2.1 Metabolic Pathways for Omega-6 and Omega-3 fatty acids**




As precursors of highly unsaturated fatty acids (HUFAs), both LA and ALA have an impact on the n-6 LCPUFAs/n-3 LCPUFAs balance in the body (Lands, 2014; Lands & Lamoreaux, 2012; Saunders et al., 2013). Despite the different derivatives produced, LA and ALA undergo similar metabolic processes and employ the same set of desaturases and elongases (Figure 2.1). Numerous studies have shown that delta-5 desaturase and delta-6 desaturase are key rate-limiting enzymes in the synthesis of HUFAs through both the n-6 and n-3 pathways (Husted & Bouzinova, 2016; Lands, 2014; Lands & Lamoreaux, 2012).

They introduce double bonds specifically in the dehydrogenation reactions of LA and ALA to ultimately yield ARA, DHA, and EPA, working in conjunction with other elongases (Husted & Bouzinova, 2016). Because the rate-limiting enzymes have a higher affinity for n-3 substrates than n-6 substrates, they show a higher preference for n-3 derivatives during the conversion process (Husted & Bouzinova, 2016). Some studies indicate that vegans and omnivores have similar ALA intake, but vegans consume little EPA and DHA (Bakaloudi et al., 2021; Saunders et al., 2013). Only 2-10% of dietary ALA is converted to EPA and DHA (Wijendran & Hayes, 2004a). The competition of LA and ALA for the same enzymes during the conversion process affects the levels of ARA, EPA, and DHA in tissues. Additionally, the processes of storage and release of ARA, EPA, and DHA in tissues also influence the competition between n-3 and n-6 fatty acids (Lands, 2014).

## 2.4 Food Sources of n-3 and n-6 Polyunsaturated Fatty Acids

### 2.4.1 Main n-3 LCPUFA Food Sources

For omnivores, the best food source of EPA and DHA is deep-sea fish. Salmon, anchovies, mackerel, and tuna are all high in n-3 LCPUFA (Tur et al., 2012). The Dietary Guidelines for Americans recommend that adults eat two 3-ounce servings of fish per week to meet their EPA and DHA requirements. Shrimp and oysters are also good natural sources of EPA and DHA (Tur et al., 2012).

Besides animal sources, a few ALA-rich terrestrial plants and deep-sea plants with EPA and DHA content are other sources of n-3 PUFAs. Plants such as chia, flax, perilla, fire hemp, algae, and walnuts are all rich in n-3 polyunsaturated fatty acids (Saunders et al., 2013). EPA and DHA food sources include marine plants such as seaweed, nori, spirulina, and chlorella (Matanjun et al., 2009; Tur et al., 2012). Seaweed and algae are good food sources for n-3 supplementation. They are also rich in micronutrients and low in fat (Mabeau & Fleurence, 1993). Vegetable oils derived from ALA-rich land plants also represent promising sources of n-3 in foods. It is efficient to increase the dietary intake of n-3 fatty acids by using high ALA oils such as flaxseed oil, chia seed oil, hempseed oil, perilla oil and tamarind oil in cooking (Asif, 2011; K. Lane et al., 2014). The nuts and seeds of walnuts, pumpkin seeds and sunflower seeds are also a major food source of n-3 LCPUFA (Simopoulos, 2002). In addition, fruits and vegetables such as spinach, brussels sprouts, amaranth, perilla and avocados, which are often found in salads are also rich in ALA (Asif, 2011; Simopoulos, 2004; Uddin et al., 2014).

### 2.4.2 Main n-6 LCPUFA Food Sources

Linoleic acid (LA) accounts for approximately 90% of dietary LCPUFA sources and is the

most common n-6 LCPUFA. (Whelan & Fritsche, 2013). In addition, LA is a precursor compound of n-6 LCPUFA (Whelan & Fritsche, 2013). Many food groups such as vegetable oils, meats, nuts, eggs and dairy products are rich in LA (Astorg et al., 2004). Fruits and vegetables also contribute to LA intake. Most vegetable oils, including peanut, corn, and sunflower oils, are abundant in linoleic acid (Fritsche, 2014). Over the past century, there has been a surge in linoleic acid consumption in the Western diet, primarily due to the widespread use of vegetable oils like soybean, corn, and sunflower oils in processed and fast-food products (Blasbalg et al., 2011).

Specific green leafy vegetables and nuts, together with borage seed, blackcurrant seed, and evening primrose, contain gamma-linolenic acid (GLA) or γ-linolenic acid (Charnock et al., 1994; Fan & Chapkin, 1998). Under healthy conditions, the human body can synthesise the required amount of GLA. However, imbalanced nutrition or a compromised physical condition may impact GLA synthesis, necessitating obtaining GLA from dietary sources (Kapoor & Huang, 2006; Chang et al., 2010).

Vegans can synthesise ARA from LA-rich plant-based foods. However, n-3 fatty acids compete and affect the conversion of ARA to LA (Tallima & El Ridi, 2018; Wiktorowska-Owczarek et al., 2015). Conjugated linoleic acid (CLA) is a naturally occurring trans-fat. It has potential health benefits including reducing cancer risk and promoting weight loss. (Ghazani & Marangoni, 2016; Nunes & Torres, 2010). Conjugated linoleic acid (CLA) is mainly formed through microbial fermentation of PUFA and linoleic acid in the digestive tract of ruminants. Therefore, foods of ruminant origin contain CLA. (Nunes & Torres, 2010). A moderate intake of n-6 LCPUFA food can reduce the risk of cardiovascular disease, benefit brain health, and improve reproductive health (Saunders et al., 2013).

## 2.5 Appropriate Ratio of n-6 and n-3 Polyunsaturated Fatty Acids

It is challenging to determine the optimal ratio of the n-3 and n-6 fatty acid families in the human diet. Ten thousand years ago, the human diet maintained a balanced nutritional state of n-3 to n-6 in a 1:1 ratio (Simopoulos, 2002d, 2016d; Simopoulos & Cleland, 2003). Although modern humans' genetics are fundamentally similar to those of the Neolithic era, there have been significant changes in diets and the food environment. With a drastic population increase, limited wild animal and plant resources could no longer meet people's energy demands. Technological innovations in agricultural cultivation and livestock farming in modern society have altered dietary patterns (Simopoulos, 2002d). Wheat, corn, rice, and some grains rich in N-6 fatty acids have gradually become the primary food types for people (Simopoulos, 2002d). Additionally, due to advancements in food processing technology and the widespread adoption of industrialised cultivation and farming techniques, plant-based

oils like soybean oil, sunflower seed oil, and peanut oil have become the main cooking oils in households. The intake of meat from domestically raised animals, which is predominant in the modern human diet, has also increased. Modern human diets have disrupted the balanced n-3/n-6 ratio observed in early human diets due to increased N-6 intake and changes in dietary structure. The established dietary ratio has shifted from 1:1 to the current 20:1 or even higher (Simopoulos, 2002d, 2016d).

Simopoulos (2002b) suggests that the ideal ratio of n-6 to n-3 LCPUFAs should be between 2:1 and 4:1, or even lower. The appropriate ratio of n-6/n-3 from food sources is relevant to the situation. A 2-3:1 ratio helps to reduce inflammation in patients with rheumatoid arthritis (Simopoulos, 2006). According to Simopoulos (2006), an n-6 to n-3 ratio of 5:1 improved asthma patient, while a ratio of 10:1 had a negative effect (Simopoulos, 2006). In patients with colorectal cancer, n-6/n-3 ratios of 2.5:1 were associated with reduced proliferation of rectal cancer cells, but there was no observed correlation if ratios were greater than 4:1 (Simopoulos, 2006). Overall, the 1:1 to 2:1 ratio target meets the results of current studies on diet, neurodevelopment, and genetic evolution. Depending on the severity of their chronic disease, Simopoulos (2006) suggests that patients increase their consumption of n-3 meals to the greatest extent possible to decrease the n-6 LCPUFAs/n-3 LCPUFAs ratio.

N-3 and N-6 fatty acids have similar and parallel breakdown and conversion mechanisms in tissues, and it is advisable to maintain similar levels of intake for both. Because it's hard to cut down on n-6 fatty acids in modern diets, nutritionists suggested eating more n-3 fatty acid-rich foods to lower the ratio of n-6 LCPUFAs to n-3 LCPUFAs (Lands & Lamoreaux, 2012; Simopoulos, 2002, 2006). When the diet is rich in ALA and has lower levels of LA consumption, there is increased EPA and DHA in muscle tissue due to competition for the Δ6 desaturase enzyme. The hydrogenation process in plant oil production leads to high concentrations of linoleic acid and reduced levels of ALA (Simopoulos, 2002a). Animal experiments conducted by Ibrahim et al. (2018) also demonstrated that the ratio of n-6 LCPUFAs to n-3 LCPUFAs in fish oil is superior to that in plant oil. Therefore, vegans should be cautious about the potential negative impact of vegetable oil intake on the n-6 LCPUFAs to n-3 LCPUFAs ratio.

## 2.6 Impact of high n-6/n-3 LCPUFAs Ratio Diets on Health Outcomes

Polyunsaturated fatty acids from dietary sources are essential in the brain, cardiovascular, and metabolic mechanisms. Antagonistic effects of n-3 and n-6 release contrasting derived signalling molecules in tissues (Burdge & Wootton, 2002; Saunders et al., 2013; Simopoulos,

2016d). When excess n-6 is consumed in the diet, the n-6/n-3 ratio does not reach a dynamic balance and contributes directly or indirectly to the pathogenesis of many diseases (Simopoulos, 2002). The competitive relationship between the two fatty acid groups affects n-3 metabolism and derives inflammatory signalling molecules (Calder, 2006). According to Hussein (2013), dietary LCPUAs affect the fatty acid composition of the human cell membrane. Phospholipase A2 binds to fatty acids on cell membranes to synthesise signalling molecules. As ARA and EPA are structurally similar, the type of fatty acids mobilised by phospholipase depends on the proportion of fatty acids on the cell membrane. Take a high n-6/n-3 Western diet as an example. The cell membrane phospholipid ratio in long-term Western diets consists of 20% ARA and 1% EPA (Calder, 2006). As the primary substrate of the eicosanoid family, ARA synthesises prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) under the catalysis of cyclooxygenase (COX) and lipoxygenase (LOX) and other inflammatory mediators (Anderson & Ma, 2009; Calder, 2006). Correspondingly, competition with n-6 after n-3 enters the body blocks arachidonic acid synthesis. In addition, N-3 increases the expression of anti-inflammatory genes by activating the anti-inflammatory transcription factor PPAR-γ and inhibiting the pro-inflammatory transcription factor NFκB to decrease the expression of pro- inflammatory genes (Calder, 2006; Kang & Weylandt, 2008). A high ratio of n-6/n-3 in the diet may be strongly associated with neurodegenerative diseases like Alzheimer's, cognitive impairment, cardiovascular diseases, and cancer, especially colon, breast, and prostate cancers.

Animal studies have demonstrated that a high n-6/n-3 ratio diet is associated with a high risk of developing Alzheimer's disease (Arsenault et al., 2011; Lim et al., 2005). Different n-6/n-3 ratio diets were given to Alzheimer's disease models in mice. Cortical Aß patch burden was higher in mice fed a high n-6/n-3 ratio diet than in mice fed a low ratio diet (Lim et al., 2005). A low n-6/n-3 ratio diet generally reduced plaque burden in mice. It also had a significant effect on plaque reduction in their hippocampus and parietal cortex. Furthermore, a study by van Elst et al. (2019) suggests that a long-term high n-6/n-3 diet is detrimental to brain development in mice. This diet pattern may increase their risk of developing neurodevelopmental disorders. Some of the available human studies support a positive correlation between dietary n-6/n-3 ratios and brain health risk (Loef & Walach, 2013). Beydoun et al. (2008) analysed the association between dietary n-6/n-3 LCPUFAs and low cognitive performance in a cross- sectional study. There was a particularly strong association between high intake levels of n-6 LCPUFAs and cognitive dysfunction in hypertensive patients. Researchers need to conduct more studies to find the optimal n-6/n-3 fatty acid ratio for brain health. Consuming diets rich in n-6 fatty acids certainly has negative health consequences, while eating a diet high in n-3 fatty acids promotes healthy brain development (Jicha & Markesbery, 2010; Loef & Walach, 2013).

Secondly, populations with a highly unbalanced n-6/n-3 ratio in their diet may be associated with a high risk of cardiovascular disease (Simopoulos, 2008). In contrast, increased n-3 consumption and a lower n-6/n-3 ratio in the diet moderate cardiovascular disease. A 4:1 n-6/n-3 ratio is even associated with a 70% reduction in cardiovascular disease mortality (Simopoulos, 2006). In studies of cardiac interventions with low n-6/n-3 ratios in the Mediterranean as a dietary intervention, the case group had significantly lower rates of cardiac mortality and non-fatal myocardial infarction compared to the control group (de Lorgeril et al., 1994; de Lorgeril & Salen, 2000). In a dietary intervention in the Lyon Heart Study (de Lorgeril et al., 1994) using olive oil instead of corn oil in participants with a single previous myocardial infarction, the overall mortality rate in these participants was reduced by 70% after two years. In a Japan Public Health Center-based (JPHC) study, Hamazaki (2018) and colleagues found that the n-6/n-3 ratio may be positively associated with the risk of cardiovascular disease.

While LA affects LDL-C metabolism in blood lipids, EPA and DHA in the n-3 family are associated with lower triglycerides (Wijendran & Hayes, 2004). In a comparative study by Valsta et al. (1992), 30 women and 29 men underwent a test in which they consumed a diet containing rapeseed or sunflower oil over 3.5 weeks, and their blood fatty acid profiles were compared. The researchers found that a diet with a low n-6/n-3 ratio of rapeseed oil was associated with lower LDL cholesterol levels than a diet with a high n-6/n-3 ratio of sunflower oil. Both diets lowered participants' total serum cholesterol levels to some extent and maintained HDL cholesterol levels consistent with baseline levels. In addition, subjects on the sunflower oil diet with a high n-6/n-3 ratio had greater platelet aggregation (Mutanen et al., 1992). It can lead to the formation of blood clots in the blood vessels, which can lead to heart disease, stroke, and blood embolism in the lungs.

Thirdly, n-6 promotes the growth of malignant tumours, whereas n-3 has a hindering effect (Reddy, 1988). In colon cancer cell studies (Berquin et al., 2008; Reddy & Sugie, 1988), a low proportion of LCPUFA intake was inversely associated with the incidence of colon cancer compared to diets with high n-6/n-3 ratios. Excessive intake of n-6 fatty acids sends a message to genes to produce more of the pro-cancer protein rasp21, which is likely to cause cancer when levels of rasp21 are chronically high in the body. A sufficient amount of n-3 fatty acids entering the body has the opposite effect on the activation of Ras. In addition, n-3 can also reduce the growth and division of cancer cells by down-regulating EPA synthesis through carboxylase II, thus significantly reducing the incidence of cancer (Larsson et al., 2004). A diet with a high n-6/n-3 ratio may significantly promote tumour growth when tumours are already present. Reddy and Sugie (1988) administered a diet of herring oil and corn oil to male F344 rats with colon cancer. At the end of the experiment, mice on a diet high in

herring oil and low in corn oil had a reduced rate of colon cancer cell proliferation. In contrast, the reproduction rate of cancer cells was higher in the high corn oil low herring oil group with a high n-6/n-3 ratio. In breast cancer cell studies, researchers have also observed an inhibitory effect of low n-6/n-3 ratio diets on cancer cells (Karmali et al., 1984; Rose & Connolly, 1993). While the number of tumour cells and prostaglandin synthesis tended to decrease in the group with a low dietary n-6/n-3 ratio, the spread of cancer cells was more severe in the experimental group of mice fed a high n-6/n-3 ratio diet. Rose and Connolly (1993) fed test mice carrying human breast cancer cells with different ratios of fatty acids. After 12 weeks, compared to subjects fed a diet containing 2% linoleic acid, the spread of cancer cells was more severe in mice fed a diet containing 2% linoleic acid. 8% and 12% linoleic acid-fed mice had more significant and heavier breast cancer tumour growth. The consumption of the high n-6/n-3 diet was also causing a more severe spread of cancer cells in the mice. The results of the current animal studies support the positive effect of n-3 in preventing the reduction of colon, prostate, and breast cancers (Karmali et al., 1984; Reddy & Sugie, 1988; Rose & Connolly, 1993). However, the available observational studies in humans have not established a correlation between the n-6/n-3 ratio diet and cancer (Marventano et al., 2015). The current inconsistent results observed in animal studies versus human studies on the effects of n-6/n-3 diet on cancer may be related to the influence of complex variables on outcomes in human clinical studies.

Finally, high n-6/n-3 consumption may also be associated with mental disorders, metabolic syndrome, obesity, and the three types of diseases stated above. A study by Kaliannan et al. (2019) on animals found that metabolic disease was more likely to happen in transgenic mice that ate a lot of n-6 and had a high n-6/n-3 ratio in their tissues. Simopoulos (2016) discovered an association between a high n-6/n-3 ratio diet and increased metabolic diseases such as diabetes and obesity in both human clinical and animal studies. A high n-6/n-3 ratio decreases insulin sensitivity in muscles, leading to an increase in body and visceral fat weight. In addition, the potential impact of dietary n-6/n-3 ratios on mental illness has also been examined. According to Husted and Bouzinova's (2016) study, increased n-6/n-3 ratios are associated with the onset of depression. People who had a high dietary n-3 intake were less likely to develop depression. When people with depression or anxiety disorders are supplemented with n-3 fatty acids and adhere to a lower n-6/n-3 diet over time, their symptoms may improve.

An unbalanced n-6/n-3 ratio may also be associated with health consequences during critical life stages, such as pregnancy and infancy. Studies on diets with high n-6/n-3 ratios during pregnancy and lactation suggest they may inhibit growth and development. The offspring of mice that maintain a high n-6/n-3 ratio in their prenatal and postnatal diets have a later onset

of physical development and puberty (van Elst et al., 2019). In the Tian et al. (2011) mince study, a lower n-6/n-3 ratio consumed by the mother during pregnancy and breastfeeding was linked to a considerable rise in PPAR-γ expression in the offspring's brains and better brain development scores. High n-6/n-3 ratios in the blood of perinatal maternal mice are associated with overweight offspring (Simopoulos, 2016). Current human clinical trials have not yet provided firm conclusions on the positive effects of a low n-6/n-3 ratio in the mother's diet on infant and child growth and development (Barnard et al., 2009; Hauner et al., 2012; Much et al., 2013). More long-term prospective studies are required to determine how reducing n-6/n- 3 intake during pregnancy and lactation affects the health of young children.

## 2.7 Intake Status and Proportions of n-6 LCPUFAs and n-3 LCPUFAs on a Vegan Diet

Vegans primarily obtain n-3 in the form of ALA in their diet (Saunders et al., 2013). While they can get sufficient ALA from plants, the conversion of ALA into EPA and DHA and the subsequent health benefits may be influenced by various factors such as sex, age, and genetics (Husted & Bouzinova, 2016; Lands, 2014; Lands & Lamoreaux, 2012). Additionally, due to the widespread presence of linoleic acid in plants and plant oils, the intake of n-6 in a vegan diet are often much higher than anticipated. Vegans tend to have a higher ratio of linoleic acid to α-linolenic acid compared to non-vegans, resulting in an elevated n-6/n-3 LCPUFAs ratio. In a study of British vegans conducted by Pinto et al. (2017), vegans exhibited an LA/ALA ratio of 13.13, while omnivores had a ratio of 10.85. In their study vegan participants also had a higher proportion of n-6/n-3 LCPUFAs in their blood fatty acid profile than omnivores.

Several studies from other countries have compared the fatty acid profiles of vegans with those of other populations. Kornsteiner et al. (2008) examined PUFA levels in the erythrocytes of Austrian vegans and omnivores. The Austrian vegans who participated in the study had a significantly higher proportion of n-6/n-3 LCPUFAs in their blood fatty acid profile than the omnivores. In the study from Mann et al. (2006), vegan participants also had a higher ratio of n-6 LCPUFAs/n-3 LCPUFAs in blood fatty acids. Menzel et al. (2022) compared the ratio of n- 6 LCPUFAs/n-3 LCPUFAs in the plasma of German vegetarians and omnivores. Plasma n-6 LCPUFAs/n-3 LCPUFA ratios were significantly higher in participants who maintained a long- term vegan diet than in omnivores. High maternal intake ratios of n-6/n-3 LCPUFAs affect their infants' fatty acid intake. The LA/ALA ratio in breast milk fat was as high as 21.1 in vegans compared to only 8.625:1 omnivores (Sanders et al.,1978). Vegan breastfeeding women also have lower levels of DHA in their breast milk

than omnivores (Sanders, 2009) (T. A. B. Sanders, 2009). It was proposed that infants of vegan mothers may be more likely to consume diets with elevated n-6 LCPUFAs/n-3 LCPUFAs ratios.

## 2.8 Intake Recommendations for n-6 and n-3 LCPUFAs

The Nutrient Reference Values for Australia and New Zealand (NRVs) released in 2006 recommended adequate intakes (AIs) for LA, ALA, and total n-3 LCPUFAs. Due to a lack of national data for New Zealand, the AI recommendations for adults were based on the median intake of relevant gender-specific Australian adults. Vegan and omnivorous adults have different dietary patterns and their food sources are different. This value may not represent the optimal intake for LA, ALA, and total n-3 LCPUFAs. Additionally, both the NRVs and the US Food and Drug Administration (FDA) have set the upper limit (UL) for total n-3 LCPUFA intake for adults at 3000 mg/day. It's worth noting that the nutrient reference values (NRVs) have also established suggested dietary targets (SDT) for preventing chronic diseases, recommending that adult males and females achieve recommended status of total n-3 LC-PUFA intake

(NHMRC, 2006). However, the NRVs do not include intake recommendations for total n-6 LC- PUFA and ARA. Table 2.1 presented n-3 and n-6 intake recommendations from several health organisations around the world. Most intake recommendations by these professional organisations are for adults based on a normal diet. Currently, no specific intake recommendations exist for n-6 and n-3 UFA for vegans, and it is not possible to estimate the upper limits of intake applicable to vegans.

**Table 2.1 N-6 and n-3 PUFAs recommendations for adults**

| Country | Organisation/Author | Year | Recommendations | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | LA | ALA | EPA | DHA | DPA | Total LC n-3 (DHA/EPA/DPA) | |
| New Zealand | National Health and Medical Research Council (NHMRC) | 2006 | Male: AI - 13 g/day<br>Female: AI - 8g/day<br>Male: AI - 12~17g/day | Male: AI -<br>Female: AI - 0.8g/day<br>Male: AI - 1.2~1.6g/day | /<br>/<br>/ | /<br>/<br>/ | /<br>/<br>/ | Male: AI - 160mg/day UL - 3000mg/day<br>Female: AI - 90mg/day UL - 3000mg/day<br>/ | National Health and Medical Research Council (NHMRC), (2006) |
| United States | Institute of Medicine (IOM) | 2002 | Female: AI - 10~12 g/day | Female: AI - 1.0~1.1g/day | / | / | / | / | Institute of Medicine (IOM), (2002) |
| French | French Food Safety Agency (ANSES) | 2014 | 4g/day | 1g/day | 250mg/day | 250mg/day | / | EPA+DHA ≥500mg/day | Philippe Legrand, (2014) |
| International | International Society for the Study of Fatty Acids and Lipids (ISSFAL) | 2000 | AI - 4.44g/day | 2.22g/day | ≥220mg/day | ≥300mg/day | / | EPA+DHA ≥650mg/day | Simopoulos et al., (2000) |

*__Abbreviations__: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; AI, Adequate Intake; UL, upper tolerable intake level

## 2.9 N-3 Supplements

Several n-3 supplements on the market help people achieve a balanced ratio of n-3 and n-6 in their diets to address potential gaps in these important nutrients. Due to the distinct sources of plant-based foods compared to omnivorous diets, vegans face unique challenges in obtaining sufficient n-3 and n-6 fatty acids from supplements.

Common n-3 supplements for vegans primarily include supplements derived from algae, flaxseed oil supplements, and other plant-based alternatives. Algae supplements produced in closed fermentation facilities have lower susceptibility to marine pollutants compared to traditional n-3 supplements like fish oil (Sun, 2018; Turner, 2006; Breivik, 2007). The bioavailability of n-3 from algal oil is high. Small doses of algal oil supplemented for short term periods were associated with an increase in blood and plasma DHA concentration (Arterburn et al., 2007; Sanders et al., 2006; Geppert et al., 2006). Studies have also found that n-3 supplements derived from flaxseed oil, chia seed oil and other plants are effective in increasing blood concentration of ALA (Lane et al., 2013).

There are no studies which compare the difference in bioavailability of n-3 between dietary and supplemental sources. The bioavailability and suitability of n-3 dietary supplements for vegans require a meticulous analysis based on peer-reviewed research.

## 2.10 Summary

N-3 and n-6 fatty acids are both essential polyunsaturated fatty acids. To meet dietary needs and maintain normal functions, it is crucial to consistently obtain an adequate amount of both n-3 and omega-6. N-6 is widely present in many food categories, whereas the sources of n-3 for vegans are relatively limited. They primarily obtain n-3 from ALA found in plants like flaxseeds and chia seeds, lacking direct intake of DHA and EPA. Ideally, the n-6 to n-3 intake ratio should be 1:1. Considering the shift in food supply from the Palaeolithic era to modern diets, a ratio of 2:1 to 4:1 for n-6/n-3 is considered to best maintain a balance between the two. However, the modern dietary patterns in many countries are characterised by a high n-6/n-3 ratio. The vegan diet is far from having a low n-6/n-3 ratio due to the inclusion of high intakes of vegetable oil consumption and less direct intake of EPA and DHA.

There is limited research evidence from available clinical studies exploring the relationship between dietary n-6 and n-3 fatty acid ratios and disease. Most studies have focused solely on the beneficial effects of n-3 in disease treatment without definitively establishing the association between n-6/n-3 and health risks. Research has been increasingly focusing on the positive effects of balanced n-6/n-3 intake ratios on brain disease, cardiovascular disease obesity, cancer, and other health issues, although direct damage to the body from a high n-

6/n-3 diet cannot be proven. The current scientific consensus is that n-6/n-3 intake should be balanced to reduce the impact of inflammatory responses on various body systems.

It is important to consider the ratio of n-3 to n-6 fatty acids in vegan diets. Many studies indicate that vegans are more susceptible to inadequate n-3 intake. Algae supplements, flaxseed oil, and other plant-based alternatives are available in New Zealand and offer promising options for meeting n-3 requirements on a vegan diet. However, due to the lack of research on the vegan population in New Zealand, there is a gap in our current knowledge about dietary intakes and foods sources in this population group. Therefore, an investigation of the dietary intake and food sources of n-6 and n-3 PUFAs in New Zealand vegans is timely.

# Chapter 3: Research Study Manuscript

## 3.1 Abstract

**Background:** The prevalence of people following a vegan diet is increasing in New Zealand and globally. Vegan diets are associated with higher omega-6 fatty acid and a lower omega-3 intakes which can pose health risks. Currently, there is limited evidence on the dietary intakes and food sources of the omega-3 fatty acids in the vegan diet.

**Objective:** This study aimed to investigate the dietary intake and food sources of n-3 LCPUFAs in New Zealand vegans.

**Study Methods:** This study assessed the dietary intake of 212 New Zealand vegans using a 4-day food record. The average amount of food eaten over four days was used to analysis intake of energy, total fat, polyunsaturated fatty acids (LCPUFAs), and omega-3 and omega-6 fatty acids (LCPUFAs) in 212 participants. Dietary intakes were compared with the Nutrient Reference Values. The contribution of each individual polyunsaturated fatty acid to the total PUFA intake was calculated, as well as the percentage of each n-3 PUFA in the total n-3 PUFA intake. In addition, the contribution of each food source to PUFA intake was determined by calculating the intake and percentage of different food subgroups.

**Results:** Total participants (N=212) had a mean (SD) age of 39.4 (12.4) years, and the majority (73%) of participants were 39.2 (12.6) years females. No significant sex differences were found in participants' n-3 LCPUFA, ALA, EPA, DPA, and DHA intake. Most participants had adequate dietary linoleic acid (LA) intake (59.6% of males; 77.3% of females above Adequate Intake (AI)) and adequate alpha-linolenic acid (ALA) intake (76.9% of males; 85.1% of females above AI). There were 97.4% participants who were below the AI of dietary total n-3 LCPUFA intake. Participants' food sources of total n-3 LCPUFAs (eicosapentaenoic acid (EPA) + docosapentaenoic acid (DPA) + docosahexaenoic acid (DHA)) were predominantly seaweed (66.6% EPA), nuts and seeds (69.5% DPA) and cereals (51% DHA).

**Conclusion:** The intake of polyunsaturated fatty acids, LA and ALA, was largely adequate in vegans, but most vegans were at risk of inadequate intake of n-3 LCPUFAs. Optimising the intake of foods rich in n-3 LCPUFAs in this group and focusing on dietary strategies to improve EPA, DHA and DPA intake is warranted.

**Keywords:** omega-3, omega-6, LCPUFA, vegan, food sources

## 3.2 Introduction

Vegan diets are characterised by the complete avoidance of animal products, with individuals consuming exclusively plant-based foods due to environmental, ethical and health motivations (Orlich et al., 2014; Papier et al., 2019). Vegans do not consume animal products such as meat, dairy, eggs, and honey relying exclusively on plant products and supplements for their nutrition. If well planned this dietary pattern can be associated with high dietary fibre and antioxidant intakes which are beneficial for reducing the risk of chronic diseases such as diabetes and hypertension (Wang et al., 2023).

Vegan diets have become more prevalent in recent years, with a growth in the vegan population globally and New Zealand. A longitudinal survey conducted by Milfont et al. (2021) across New Zealand showed that 1.1% of New Zealanders were vegan. Furthermore, 35% of New Zealanders who participated in the PricewaterhouseCoopers (PwC) 2019 dietary survey indicated they were interested in vegetarian and vegan diets and were likely to change their food sources (Skinner et al., 2020). Consumer interest in plant-based diets and foods has led to an increasing availability of plant-based foods in the food supply. Due to the lack of animal products vegan diets may be deficient in specific nutrients such as vitamin D, B12 and omaga- 3 long-chain polyunsaturated fatty acids lipids (n-3 LCPUFAs) (Craig, 2009). Although it is possible to consume an adequate dietary intake of alpha-linolenic acid (ALA), docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) intake may be deficient due to a lack of fish and seafood intake which are the main food sources of these nutrients (Mann et al., 2006; Sanders et al., 1978). A high intake of vegetable oils often seen in vegan diets increases n-6 LCPUFA consumption (Astorg et al., 2004; Whelan & Fritsche, 2013). Vegan diets have been characterised by higher n-6 LCPUFA intake and lower n-3 LCPUFA compared to omnivores (Kornsteiner et al. 2008; Mann et al. 2006; Pinto et al. 2017; Sanders et al. 1978; Serra-Majem et al. 2012). High dietary n-6 /n-3LCPUFA ratios are associated with a range of adverse health outcomes, including cardiovascular disease, inflammation, and cancer (Dighriri et al., 2022; Schverer et al., 2020; Simopoulos, 2008; Berquin et al., 2008; Reddy & Sugie, 1988).

Achieving n-3 dietary intake and maintaining an appropriate ratio of n-6 /n-3 LCPUFA intake is important for optimal health outcomes (Simopoulos, 2002). Although the National Health and Medical Research Council (NHMRC) and other international health organisations provided dietary intake recommendations for omega-3 LCPUFAs intakes, these recommendations usually have little specific guidance for vegans. Due to limited evidence on the n-3 LCPUFA dietary intake status in New Zealand, more knowledge of this group's dietary intake status is required. This study will fill this research gap by focusing on the dietary status of n-3 LCPUFAs in New Zealand vegans and their food sources. The intake status of

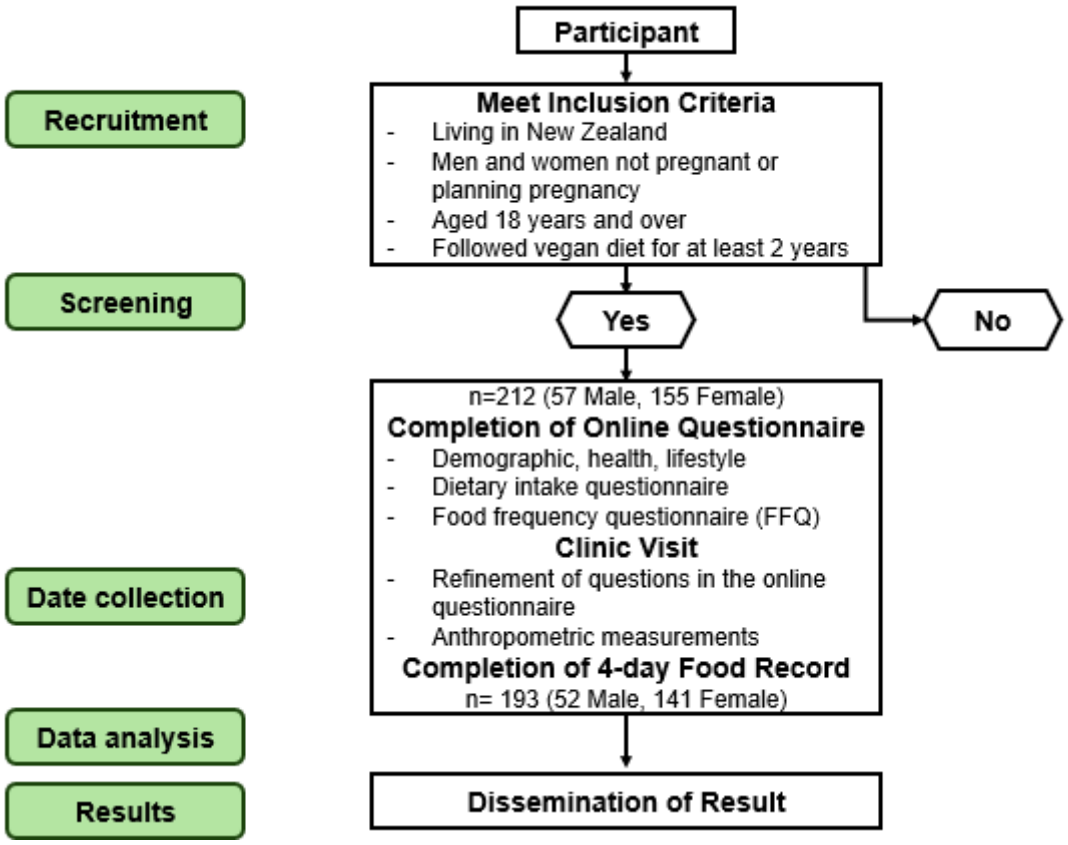
energy, total fat, LCPUFAs, and n-3 and n-6 LCPUFAs in vegans are all indicators for assessing the dietary status of a vegan diet. This study will evaluate the n-3 LCPUFAs intake status of New Zealand vegans and identify the main food sources of n-3 LCPUFAs in New Zealand vegans and their food sources. The intake status of energy, total fat, LCPUFAs, and n-3 and n-6 LCPUFAs in vegans are all indicators for assessing the dietary status of a vegan diet. This study will evaluate the n-3 LCPUFAs intake status of New Zealand vegans and identify the main food sources of n-3 LCPUFAs for vegans through a 4-day food record provided by participants.

## 3.3 Materials and Methods

This study is a partial analysis of data from the Vegan Health Research Programme conducted in Auckland, New Zealand, which is a cross-sectional study aimed to determine the dietary intake, nutritional, health and anthropometric status of people who have been on a vegan diet for 2-plus years. This specific analysis examines the omega-3 fatty acid intake and dietary sources of participants as well as the ratio of omega-6 LCPUFA to omega-3 LCPUFA in their diets. Data were collected from July 2022 to March 2023, and the flow of the participants through the study is shown in Figure 3.1.

**Figure 3.1**

*Research procedures*




Ethical approval for the study was obtained from the Health and Disability Ethics Committee (HDEC 2022 EXP 12312). The research was funded by the Lottery Health Project Grant (LHR- 2022-185693).

### Participants and recruitment

Eligible participants were adults residing in New Zealand who had adhered to a vegan diet for at least two years and were neither pregnant nor planning to become pregnant.

Recruitment was conducted via information leaflets and posters distributed on the Auckland Vegan Facebook page and specific interest groups. Appendix 2 shows the detailed screening questionnaire questions. Interested individuals completed an online screening questionnaire, and eligibility was confirmed by phone or email. There were over 500 expressions of interest, with 212 eligible participants ultimately enrolling in the study.

## Study procedures and data collection

### Demographic questions:

Participants eligible for inclusion were required to complete an online consent form and demographic questionnaire. The questionnaire consisted of seven demographic questions that participants could access and complete on the Massey University Health School website. Appendix 3 shows the specific questions of the health and demographic information questionnaire. The demographic questions collected data on participants' age, gender, education, ethnicity, and marital status. Participants were required to visit the Nutrition Clinic on the Albany campus of Massey University in Auckland, where trained researchers assisted participants to complete the questionnaires and collected anthropometric data. The researcher checked the questionnaires and gave the participant a 4-day food record form when they visited.

### Anthropometric measurements:

Anthropometric indicators were measured on-site by trained researchers. Height was measured using stadiometers, with participants standing flat-footed, heels together, and back against a wall. Measurements were accurate to one decimal place and repeated twice to ensure consistency, with a third measurement if needed. Weight was measured using an InBody device, with participants removing heavy clothing and personal items. Measurements were repeated twice for accuracy, with a third taken if necessary. Waist and hip circumferences were measured to calculate Waist–hip ratio (WHR), with measurements taken twice and a third time if discrepancies occurred.

### 4-Day Food Record (4-DFR):

Participants were asked to record in detail all the food and beverages they consumed on four consecutive days (including three weekdays and one weekend day). This dietary assessment method helps to capture a representative snapshot of participants' usual dietary intake, thus providing a comprehensive picture of vegan dietary intake and allowing for interpretation of day-to-day variability. Participants were expected to record as much detail as they could regarding each food taken across the four days: portion size, method of cooking, condiments and additives that were used, time, and place of the meal. The participants were encouraged to make such records either during or immediately after eating so as not to cause

inaccuracies in the data due to poor memory. The researchers provided each participant with detailed recording instructions and examples to help them achieve a consistent and relative representation of their diet and food intake (Appendix 4).

During the recording period, the research team kept in contact with the participants and provided them with support and guidance to ensure they completed the food diary accurately. If participants encountered problems during the recording process, they could contact the researcher for assistance at any time. At the end of the study, the research team checked each food diary to ensure data completeness and accuracy.

### Dietary Intake Assessment

After recovery of participants' 4-DFR trained researchers entered each record into FoodWorks10 (Xyris Software, Sydney, Australia) software according to standardised procedures. Some participants mentioned several currently marketed vegan products and ingredients in their 4-DFR that were not included in the database. Researchers in the vegan study group replaced foods not included in the database with similar alternatives. All replacements were carefully discussed, and a consensus was reached before making any substitutions. A research assistant checked the accuracy of all entries and assessed the nutrients. Median intake of energy, PUFA, individual n-3LCPUFAs (ALA, DHA, EPA), individual n-6LCPUFAs, have been compared against the Estimated Average Requirement (EAR), Adequate Intake (AI) recommended for New Zealand adults (National Health and Medical Research Council, 2006). This study also collected data on participants' n-3 LCPUFAs supplement use. The median intake of polyunsaturated fatty acids (n-3LCPUFAs and n-6LCPUFAs) were compared to the nutrient reference values (NRVs). The percentage of participants meeting the EAR, or AI (in the absence of an EAR) for LA, ALA and total n-3 LCPUFA were calculated. This study defines "inadequate intake" as less than EAR.

## Statistical Analysis

This study used SPSS statistical software for statistical analyses (version 27.0; SPSS Inc., Chicago, IL).

Descriptive statistics were used for participants' sex, age, ethnicity, highest level of education, smoking status, medications use and dietary supplement use.

Normality plots and the Kolmogorov-Smirnov test were calculated to see if the age distribution, kilojoule intake, PUFA, individual n-3LCPUFAs (including ALA, DHA, and EPA), and individual n-6LCPUFAs dietary intake were all normal. The mean ± standard deviation (SD) was used to depict the data for variables that follow a normal distribution. However, the median (25th, 75th percentile) was employed for variables that do not follow a normal distribution. The logarithmic transformation was employed to convert data that did not follow

a normal distribution into geometric means accompanied by 95% confidence intervals. Statistics were subsequently verified to ensure their conformity with expectations.

To determine ethnic and sex differences in dietary intake of energy, PUFA, individual n-3LCPUFAs (ALA, DHA, EPA), and individual n-6LCPUFAs among vegans, the researcher used the Mann-Whitney U-test and independent samples t-test. In addition, the researcher used a paired-sample t-test, Pearson chi-square test, and one-way ANOVA (one-way ANOVA) for data processing and analysis.

## Food source analysis

4-day Food Record was used to calculate the proportions and contributions of different food sources to the total daily intake of PUFAs. The proportion of each food source to the total and individual PUFA intake was expressed as mean grammes per day and then calculated as the percentage of each PUFA intake to the mean intake of the total sample. These averages do not include the contribution of PUFA supplementation. Further analyses determined the proportion and contribution of each food group to total PUFA, individual n-3LCPUFAs (ALA, DHA, EPA), and individual n-6 LCPUFAs.

To ensure the justice of the analysis, the researcher used standardised procedures and strict quality control measures. Training researchers reviewed and entered all food records using uniform dietary analysis software (Food Works10). It excluded any possible effects of supplements to ensure the accuracy and consistency of the results.

Detailed descriptions and procedures were provided for this study's food records and data analysis sections. These ensured that the analyses were transparent and reproducible, allowing other researchers to use these descriptions to repeat the analyses and verify the reliability of the results.

## 3.4 Results

### 3.4.1 Participant Characteristics

The mean (SD) age of the participants in this study was 39.4 (12.41) years. 73% of the participants were female (n=155). Four participants reported their gender as diverse. This study only reported sex and no other categories, so they were re-categorised by biological sex. Most participants were European (84.8%). More than half of the participants (68.9%) had a bachelor's degree or higher as their highest level of education. Table 3.1 presents the demographic of the participants (n=212).

**Table 3.1 Demographic description and health assessment results of participants**

| Participants characteristics | | Total | Male | Female | p-value1 |
|---|---|---|---|---|---|
| **Number of participants, n (%)** | | 212 | 57 (27%) | 155 (73%) | **<0.01** |
| **Age (years)** | | | | | 0.55 |
| | Mean (SD) | 39.43 (12.41) | 40.12 (12.11) | 39.18 (12.55) | |
| | Median (IQR) | 37.70 (28.75, 49.15) | 38.20 (31.80, 49.90) | 36.60 (28.40,48.85) | |
| | Range | 19.70, 75.60 | 21.40, 69.30 | 19.70, 75.60 | |
| **Ethnicity, n (%)** | | | | | 0.11 |
| | European | 178 (84.8%) | 49 (87.5%) | 129 (83.8%) | |
| | Māori | 8 (3.8%) | 0 (0%) | 8 (5.2%) | |
| | Pacific Peoples | 1 (0.5%) | 1 (1.8%) | 0 (%) | |
| | Asian | 16 (7.6%) | 3 (5.4%) | 13 (8.4%) | |
| | Middle Eastern/Latin American | 7 (3.3%) | 3 (5.4%) | 4 (2.6%) | |
| | Missing | 2 | 1 | 1 | |
| **Highest Education Level, n (%)** | | | | | **0.015** |
| | Lower than high school | 3 (1.4%) | 1 (1.8%) | 2 (1.3%) | |
| | High school | 18 (8.5%) | 7 (12.3% | 11 (7.1%) | |
| | Dipolma/Certificate | 45 (21.2%) | 18 (31.6%) | 27 (17.4%) | |
| | Bachelor’s degree | 95 (44.8%) | 14 (24.6%) | 81 (52.3%) | |
| | Master’s degree | 40 (18.9%) | 13 (22.8%) | 27 (17.4%) | |
| | Doctoral level | 11 (5.2%) | 4 (7.0%) | 7 (4.5%) | |
| **Length of time vegan, n (%)** | | | | | 0.51 |
| | 2-4 years | 84 (40%) | 22 (39%) | 62 (40%) | |
| | 5-10 years | 93 (44%) | 28 (49%) | 65 (42%) | |
| | 10+ years | 35 (17%) | 7 (12%) | 28 (18%) | |
| **WC** | Mean (SD) | 81.84 (10.56) | 87.54 (8.56) | 79.74 (10.47) | <0.001 |
| | Median (IQR) | 81.00 (74.83, 88.30) | 86.60 (82.60, 92.90) | 78.00 (71.93, 85.85) | |

| | | | | | |
|---|---|---|---|---|---|
| | Range | 59.50, 124.50 | 70.45, 109.50 | 59.50, 124.50 | |
| **HC** | Mean (SD) | 97.47 (7.85) | 97.55 (7.70) | 97.44 (7.93) | 0.94 |
| | Median (IQR) | 96.70 (92.33, 102.28) | 97.15 (92.00, 103.10) | 96.70 (92.50, 101.73) | |
| | Range | 76.00, 131.75 | 82.45, 118.50 | 76.00, 131.75 | |
| **BMI** | Mean (SD) | 23.92 (3.11) | 24.48 (2.97) | 23.71 (3.15) | 0.08 |
| | Median (IQR) | 23.72 (21.73, 25.51) | 24.36 (22.86, 26.07) | 23.53 (21.52, 25.42) | |
| | Range | 16.35, 36.06 | 18.50, 33.24 | 16.35, 36.06 | |

**1Wilcoxon rank sum test; *Missing data**

*** Abbreviation:** WC, Waist circumference, average of 2 measures, (cm); HC, Hip circumference, average of 2 measures, (cm); BMI, Body Mass Index (kg/m2).

### 3.4.2 Energy and Dietary Fatty Acids Intakes

As the dietary intake data was not evenly distributed, the median (IQR) values were utilised to define the participants' PUFA intake and the central tendency of the data distribution.

It is important to note that there were missing data points in the analysis. There were 19 instances of missing data, with five missing from male participants and 14 from female participants. Overall, male vegans had significantly higher energy intake (11,304.55 kJ/d) compared to female vegans (8,256.02 kJ/d) ($p < 0.001$). Similarly, the total fat intake was higher in male vegans (105.04 g/d) than in female vegans (80.17 g/d) ($p < 0.001$). The intake of polyunsaturated fatty acids (PUFAs) was also higher in males (28.21 g/d) compared to females (20.90 g/d) ($p < 0.001$). Male vegans had a lower intake of linoleic acid (LA) (16.29 g/d) compared to female vegans (12.66 g/d) ($p = 0.001$).

Since total n-6 and n-3 values were unavailable, the essential n-3 and n-6 fatty acids were used in this table, and ratios were calculated based on these data. Table 3.2 details the dietary intake of total and individual PUFAs for male and female vegan participants over four days.

**Table 3.2 Daily dietary intakes of energy and polyunsaturated fatty acids**

| **Characteristic** | **Overall, N = 193** | **Male, N = 46** | **Female, N = 141** | **p- value1** |
|---|---|---|---|---|
| **EnergyDF_kJ** | | | | **<0.001** |
| Mean (SD) | 9,077.39 (2,519.25) | 11,304.55 (2,652.36) | 8,256.02 (1,902.45) | |
| Median (IQR) | 8,719.14 (7,084.87, 10,446.69) | 11,092.95 (9,697.88, 12,393.25) | 7,920.64 (6,904.93, 9,361.36) | |
| Range | 3,169.77, 18,907.53 | 6,503.23, 18,907.53 | 3,169.77, 13,504.17 | |
| **Saturated_fat_g** | | | | **<0.001** |
| Mean (SD) | 21.47 (10.28) | 24.93 (10.45) | 20.20 (9.95) | |

| | | | | |
|---|---|---|---|---|
| Median (IQR) | 20.07 (14.13, 26.22) | 23.61 (18.94, 28.77) | 19.30 (13.20, 24.45) | |
| Range | 4.03, 65.91 | 5.42, 65.91 | 4.03, 65.12 | |
| **Polyunsaturated_fat_g** | | | | **<0.001** |
| Mean (SD) | 22.99 (8.89) | 28.65 (9.09) | 20.90 (7.87) | |
| Median (IQR) | 21.53 (16.14, 28.30) | 28.21 (21.39, 34.94) | 19.26 (15.43, 24.61) | |
| Range | 3.52, 55.39 | 13.59, 54.28 | 3.52, 55.39 | |
| **VLC_N3_g** | | | | 0.28 |
| Mean (SD) | 0.01 (0.02) | 0.01 (0.01) | 0.01 (0.03) | |
| Median (IQR) | 0.01 (0.00, 0.01) | 0.00 (0.00, 0.01) | 0.01 (0.00, 0.02) | |
| Range | 0.00, 0.20 | 0.00, 0.07 | 0.00, 0.20 | |
| **LA__g** | | | | 0.001 |
| Mean (SD) | 13.64 (6.39) | 16.29 (7.24) | 12.66 (5.77) | |
| Median (IQR) | 12.57 (9.06, 16.75) | 14.42 (11.28, 21.19) | 12.05 (8.59, 15.60) | |
| Range | 1.34, 32.78 | 5.75, 32.78 | 1.34, 32.69 | |
| **ALA_g** | | | | 0.088 |
| Mean (SD) | 2.27 (1.69) | 2.46 (1.53) | 2.20 (1.75) | |
| Median (IQR) | 1.79 (1.12, 2.91) | 2.22 (1.37, 3.28) | 1.68 (1.06, 2.91) | |
| Range | 0.06, 9.52 | 0.17, 6.12 | 0.06, 9.52 | |
| **EPA_g** | | | | 0.18 |
| Mean (SD) | 0.01 (0.02) | 0.00 (0.01) | 0.01 (0.03) | |
| Median (IQR) | 0.00 (0.00, 0.01) | 0.00 (0.00, 0.00) | 0.00 (0.00, 0.01) | |
| Range | 0.00, 0.20 | 0.00, 0.05 | 0.00, 0.20 | |
| **DPA_g** | | | | 0.13 |
| Mean (SD) | 0.00 (0.01) | 0.00 (0.01) | 0.00 (0.00) | |
| Median (IQR) | 0.00 (0.00, 0.01) | 0.00 (0.00, 0.01) | 0.00 (0.00, 0.00) | |
| Range | 0.00, 0.03 | 0.00, 0.03 | 0.00, 0.02 | |
| **DHA_g** | | | | 0.16 |
| Mean (SD) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | |
| Median (IQR) | 0.00 (0.00, 0.00) | 0.00 (0.00, 0.00) | 0.00 (0.00, 0.00) | |
| Range | 0.00, 0.03 | 0.00, 0.01 | 0.00, 0.03 | |
| **LA/ALA ratio** | | | | 0.41 |
| Mean (SD) | 8.47 (5.82) | 8.53 (5.86) | 8.43 (5.81) | |
| Median (IQR) | 6.92 (5.01,10.91) | 6.93 (5.08,10.93) | 6.84 (4.99,10.77) | |
| Range | 1.10,41.73 | 1.10,41.73 | 1.10,41.73 | |
| **n-6/n-3 ratio** | | | | 0.4 |
| Mean (SD) | 8.40 (5.77) | 5.46 (5.80) | 8.36 (5.75) | |
| Median (IQR) | 6.71 (5.00,10.78) | 6.78 (5.07,10.80) | 6.70 (4.98,10.74) | |
| Range | 1.10,41.73 | 1.10,41.73 | 1.10,41.73 | |

**1)Wilcoxon rank sum test; 2) 19 Missing data: 5 male and 14 female missing**

**3) the intakes for EPA, DPA, and DHA are displayed as zero due to rounding to two decimal places. These values are not actually zero but are very small amounts that, when rounded, appear as zero. Despite this rounding, the omega-6/omega-3 ratios were calculated using the actual values before rounding, allowing for a valid and non-zero ratio calculation.**

* **Abbreviation**: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; VLCN3, DPA+DHA+EPA; n-6, omega-6 long chain polyunsaturated fatty acid; n-3, omega-3 long chain polyunsaturated fatty acid

### 3.4.3 Intake and Adequacy of Omega-3 and Omega-6

The NHMRC recommends a daily intake of 160 mg of total n-3 long-chain PUFA (EPA+DPA+DHA) for men and 90 mg for women. Due to missing data, only five female participants (3.5%) met this AI recommendation. No participants exceeded the NHMRC upper intake limit of 3000 mg/day for n-3 long-chain PUFA. Participants' total n-3 long-chain PUFA intake ranged from 0.03 mg to 202.01 mg/day, showing no significant differences between male and females. The participants' total intake of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) compared to recommendations are shown in Table 3.3.

**Table 3.3 Comparison of participants' intake of omega-3 and omega-6 polyunsaturated fatty acids with recommendations**

| | Dietary intakes | | n (%) meeting recommendations | |
|---|---|---|---|---|
| | Male, N = 52 | Female, N = 141 | Male | Female |
| LA_g/d<br>AI recommendation Males - 13g/d Females - 8g/d | 14.42 (11.28, 21.19) | 12.05 (8.59, 15.60) | 31 (59.6%) | 109 (77.3%) |
| ALA_g/d<br>AI recommendation Males - 1.3mg/d Females - 0.8mg/d | 2.22 (1.37, 3.28) | 1.68 (1.06, 2.91) | 40 (76.9%) | 120 (85.1%) |
| Total n-3 LCPUFA (EPA+DPA+DHA) _mg/d<br>AI recommendation Males - 1.3mg/d Females - 0.8mg/d<br>Ul - 3000mg/d | 5.24 (1.80, 10.38) | 7.06 (1.82, 14.47) | 0 | 5 (3.5%) |

***Missing data:** total 19 missing data, 5 male and 14 female they didn't complete the food record

**Abbreviation:** LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; VLCN3, very long-chain n-3 fatty acids, DPA+DHA+EPA; NHMRC, National Health and Medical Research Council; AI, Adequate Intake; UL, Tolerable Upper Intake Level

### 3.4.4 Contribution of Individual Fatty Acids to Total PUFA Intake

Figures 3.2 and 3.3 illustrate the contribution of different fatty acids to the participants total polyunsaturated fatty acid (PUFA) intake.

Figure 3.2 shows the contribution of each fatty acid to total dietary PUFA intake. The results show that LA contributed 59.33% of the total dietary PUFA intake. N-3 PUFAs contributed 9.93% of the total PUFA intake. ALA was the major component, accounting for 9.87%.

**Figure 3.2** Contribution of n-3 and n-6 fatty acids to total PUFA intake

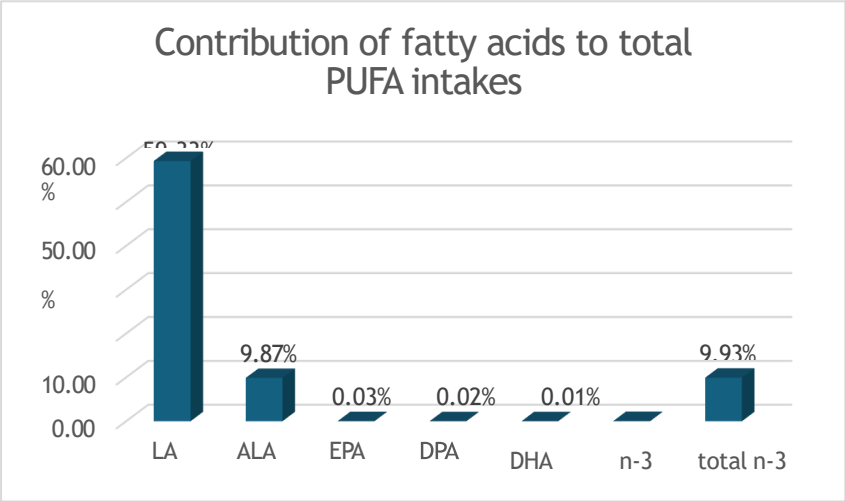



Figure 3.3 shows in further detail the proportion of ALA and each n-3 LCPUFA in the total n-3 PUFA intake. ALA comprises nearly all (99.44%) of the overall n-3 PUFA consumption, while other long-chain n-3 PUFA such as EPA (0.35%), DPA (0.15%), and DHA (0.06%) accounted for only a small percentage.

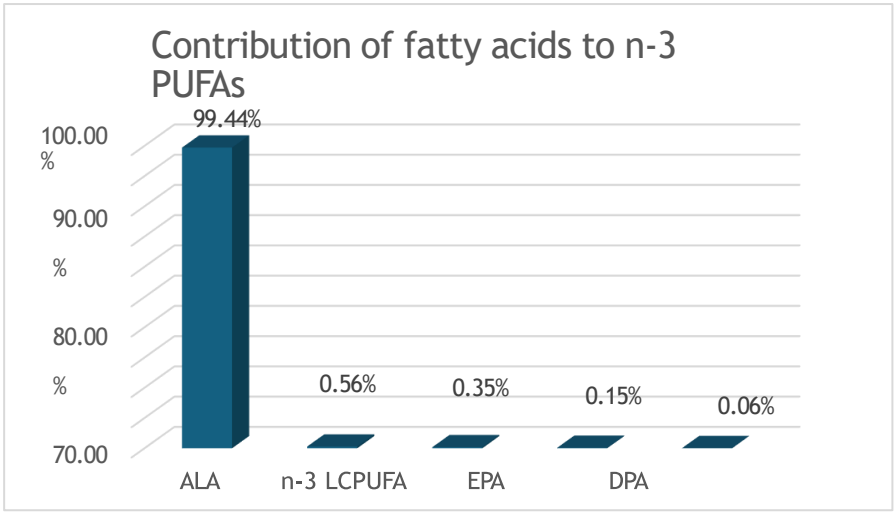



**Figure 3.3** Contribution of individual fatty acids to total n-3 PUFAs

### 3.4.5 Food Sources of polyunsaturated fatty acids

Table 3.4 details the main food sources of PUFA and their contribution to the total intake of the participants during the four days of dietary records. The main sources of PUFA were nuts and seeds (12.11%), followed by tofu (10.15%), plant-based dairy alternatives (8.56%) and fats and oils (6.80%). These food groups were also the primary sources of LA, with nuts and seeds (15.61%) being the major source, followed closely by imitation dairy products (11.77%), tofu (9.89%) and fats and oils (7.92%).

The main food sources of ALA were nuts and seeds (23.96%), bread and crackers (11.06%) and tofu (9.54%). Vegetables accounted for a large proportion of EPA (37.98%) intake, followed by savoury snacks (2.44%) and sweet snacks (2.30%). The main sources of DPA were nuts and seeds (69.51%), followed by fruits (4.58%), tofu (2.93%), and breakfast foods (2.33%). On the other hand, the main sources of DHA were cereals (22.61%) and bread and biscuits (13.29%), while desserts, fats and oils, nuts and seeds, fruits and imitation dairy products contributed less to DHA.

**Table 3.4 - Contribution of food sources to n-6 and n-3 polyunsaturated fatty acid intake**

| Food source | Total PUFA | | LA | | ALA | | VLC_N3 | | EPA | | DPA | | DHA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g | % | g | % | g | % | g | % | mg | % | mg | % | mg | % |
| **Condiment** | **1330** | **7.56%** | **674** | **6.44%** | **56** | **3.20%** | **150.86** | **1.56%** | **23.54** | **0.39%** | **31.52** | **1.16%** | **95.8** | **9.12%** |
| Sauce | 79 | 0.45% | 46 | 0.44% | 5 | 0.29% | 67.56 | 0.70% | 20.24 | 0.33% | 4.1 | 0.15% | 43.22 | 4.11% |
| Spread | 148 | 0.84% | 80 | 0.76% | 8 | 0.46% | 10.11 | 0.10% | 0.01 | 0.00% | 0.04 | 0.00% | 10.06 | 0.96% |
| Spice | 5 | 0.03% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Salt | 1 | 0.01% | 1 | 0.01% | 0 | 0.00% | 10 | 0.10% | 0 | 0.00% | 6 | 0.22% | 0 | 0.00% |
| Dressing | 655 | 3.72% | 171 | 1.63% | 17 | 0.97% | 50.78 | 0.52% | 2.66 | 0.04% | 5.76 | 0.21% | 42.37 | 4.03% |
| Dips | 399 | 2.27% | 351 | 3.35% | 23 | 1.32% | 0 | 0.00% | 0.12 | 0.00% | 4.58 | 0.17% | 0 | 0.00% |
| Other | 39 | 0.22% | 24 | 0.23% | 2 | 0.11% | 11.55 | 0.12% | 0.52 | 0.01% | 11.03 | 0.41% | 0 | 0.00% |
| Herb | 3 | 0.02% | 1 | 0.01% | 2 | 0.11% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| **Fats and oils** | **877** | **4.98%** | **830** | **7.93%** | **103** | **5.89%** | **41.36** | **0.43%** | **41.36** | **0.68%** | **0** | **0.00%** | **0** | **0.00%** |
| Coconut | 2 | 0.01% | 2 | 0.02% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Canola Oils | 147 | 0.84% | 90 | 0.86% | 43 | 2.46% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Olive Oils | 197 | 1.12% | 181 | 1.73% | 17 | 0.97% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Seasame Oils | 40 | 0.23% | 40 | 0.38% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Sunflower Oils | 57 | 0.32% | 56 | 0.53% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Coconut | 7 | 0.04% | 6 | 0.06% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Vegetables Oil | 26 | 0.15% | 21 | 0.20% | 5 | 0.29% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Rice Oils | 66 | 0.38% | 63 | 0.60% | 2 | 0.11% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Other Oils | 5 | 0.03% | 4 | 0.04% | 1 | 0.06% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Fat Spread | 322 | 1.83% | 365 | 3.49% | 28 | 1.60% | 41.36 | 0.43% | 41.36 | 0.68% | 0 | 0.00% | 0 | 0.00% |

| Food source | Total PUFA | | LA | | ALA | | VLC_N3 | | EPA | | DPA | | DHA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seeds Oils | 10 | 0.06% | 2 | 0.02% | 8 | 0.46% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| **Vegetable** | **1328** | **7.55%** | **774** | **7.39%** | **112** | **6.41%** | **242.9** | **2.51%** | **114.16** | **1.87%** | **62.71** | **2.31%** | **66.03** | **6.28%** |
| Legumes | 313 | 1.78% | 180 | 1.72% | 32 | 1.83% | 70.23 | 0.72% | 30.53 | 0.50% | 38.05 | 1.40% | 1.66 | 0.16% |
| Avocado | 368 | 2.09% | 337 | 3.22% | 20 | 1.14% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Mushroom | 41 | 0.23% | 34 | 0.32% | 3 | 0.17% | 12.01 | 0.12% | 11.58 | 0.19% | 0.43 | 0.02% | 0 | 0.00% |
| Allium | 25 | 0.14% | 20 | 0.19% | 5 | 0.29% | 4.68 | 0.05% | 0.8 | 0.01% | 3.88 | 0.14% | 0 | 0.00% |
| Root | 93 | 0.53% | 60 | 0.57% | 10 | 0.57% | 20 | 0.21% | 1.79 | 0.03% | 6.98 | 0.26% | 8.44 | 0.80% |
| Mixed | 291 | 1.65% | 43 | 0.41% | 8 | 0.46% | 60 | 0.62% | 47.69 | 0.78% | 4.22 | 0.16% | 4.03 | 0.38% |
| Cucurbitaceae | 62 | 0.35% | 23 | 0.22% | 10 | 0.57% | 0.23 | 0.00% | 0 | 0.00% | 0.23 | 0.01% | 8.76 | 0.83% |
| Solanaceae | 48 | 0.27% | 30 | 0.29% | 4 | 0.23% | 0.29 | 0.00% | 0.05 | 0.00% | 0.24 | 0.01% | 0 | 0.00% |
| Green leaves vegetables | 91 | 0.52% | 54 | 0.52% | 15 | 0.86% | 70 | 0.72% | 21.2 | 0.35% | 6.04 | 0.22% | 43.14 | 4.11% |
| Other vegetables | 78 | 0.44% | 18 | 0.17% | 7 | 0.40% | 0 | 0.00% | 0.54 | 0.01% | 2.63 | 0.10% | 0 | 0.00% |
| **Nuts and seeds** | **3031** | **17.23%** | **2240** | **21.40%** | **623** | **35.64%** | **1950** | **20.12%** | **60.41** | **0.99%** | **1886.7** | **69.51%** | **0** | **0.00%** |
| Nuts | 1293 | 7.35% | 1109 | 10.59% | 103 | 5.89% | 10 | 0.10% | 10.11 | 0.17% | 0 | 0.00% | 0 | 0.00% |
| Seeds | 1186 | 6.74% | 668 | 6.38% | 505 | 28.89% | 50 | 0.52% | 49.2 | 0.81% | 0 | 0.00% | 0 | 0.00% |
| Nuts butter | 457 | 2.60% | 456 | 4.36% | 15 | 0.86% | 1890 | 19.50% | 1.11 | 0.02% | 1886.7 | 69.51% | 0 | 0.00% |
| Seeds butter | 96 | 0.55% | 7 | 0.07% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| **Plant-based dairy alternatives** | **1584** | **9.00%** | **1302** | **12.44%** | **49** | **2.80%** | **1.22** | **0.01%** | **0.01** | **2.70%** | **1.09** | **0.04%** | **0.12** | **0.01%** |
| Pseudo cheese | 31 | 0.18% | 17 | 0.16% | 1 | 0.06% | 0.12 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0.12 | 0.01% |

| Food source | Total PUFA | | LA | | ALA | | VLC_N3 | | EPA | | DPA | | DHA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pseudo yoghurt | 46 | 0.26% | 11 | 0.11% | 2 | 0.11% | 0.03 | 0.00% | 0.01 | 0.00% | 0.01 | 0.00% | 0 | 0.00% |
| Almond milk | 100 | 0.57% | 167 | 1.60% | 1 | 0.06% | 0 | 0.00% | 0 | 0.00% | 1.08 | 0.04% | 0 | 0.00% |
| Oat milk | 304 | 1.73% | 340 | 3.25% | 5 | 0.29% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Soy milk | 1044 | 5.93% | 709 | 6.77% | 40 | 2.29% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Coconut milk | 3 | 0.02% | 3 | 0.03% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Rice milk | 5 | 0.03% | 4 | 0.04% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Other pseudo milk | 2 | 0.01% | 9 | 0.09% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Cream | 47 | 0.27% | 40 | 0.38% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| **Fruit** | **85** | **0.48%** | **47** | **0.45%** | **15** | **0.86%** | **110** | **1.14%** | **103.31** | **1.70%** | **124.82** | **4.60%** | **1.75** | **0.17%** |
| Dried fruit | 11 | 0.06% | 7 | 0.07% | 3 | 0.17% | 80 | 0.83% | 73.02 | 1.20% | 0.39 | 0.01% | 1.75 | 0.17% |
| Jam | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Canned fruit | 22 | 0.13% | 13 | 0.12% | 2 | 0.11% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Fresh fruit | 45 | 0.26% | 20 | 0.19% | 9 | 0.51% | 30 | 0.31% | 30.26 | 0.50% | 124.35 | 4.58% | 0 | 0.00% |
| Frozen fruit | 7 | 0.04% | 6 | 0.06% | 1 | 0.06% | 0.11 | 0.00% | 0.03 | 0.00% | 0.08 | 0.00% | 0 | 0.00% |
| **Grain** | **2406** | **13.68%** | **1460** | **13.95%** | **348** | **19.91%** | **850** | **8.77%** | **189.92** | **3.12%** | **128.81** | **4.75%** | **536.18** | **51.04%** |
| Oats | 574 | 3.26% | 323 | 3.09% | 67 | 3.83% | 40 | 0.41% | 4.31 | 0.07% | 32.24 | 1.19% | 0.58 | 0.06% |
| Bread | 938 | 5.33% | 629 | 6.01% | 199 | 11.38% | 430 | 4.44% | 90.56 | 1.49% | 39.85 | 1.47% | 298.04 | 28.37% |
| Rice | 86 | 0.49% | 77 | 0.74% | 3 | 0.17% | 0.41 | 0.00% | 0.41 | 0.01% | 0 | 0.00% | 0 | 0.00% |
| Cracker | 119 | 0.68% | 51 | 0.49% | 6 | 0.34% | 40 | 0.41% | 11.47 | 0.19% | 2.42 | 0.09% | 0 | 0.00% |
| Muesli | 395 | 2.25% | 177 | 1.69% | 27 | 1.54% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Cereal | 54 | 0.31% | 50 | 0.48% | 5 | 0.29% | 50 | 0.52% | 21.81 | 0.36% | 31.09 | 1.15% | 0 | 0.00% |

| Food source | Total PUFA | | LA | | ALA | | VLC_N3 | | EPA | | DPA | | DHA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Other Grains | 68 | 0.39% | 27 | 0.26% | 2 | 0.11% | 295.95 | 3.05% | 58.39 | 0.96% | 0 | 0.00% | 237.57 | 22.61% |
| Flour | 3 | 0.02% | 2 | 0.02% | 0 | 0.00% | 1.1 | 0.01% | 0.3 | 0.00% | 0.8 | 0.03% | 0 | 0.00% |
| Wrap | 127 | 0.72% | 101 | 0.96% | 20 | 1.14% | 2.54 | 0.03% | 2.54 | 0.04% | 0 | 0.00% | 0 | 0.00% |
| Corn | 42 | 0.24% | 23 | 0.22% | 19 | 1.09% | 0.81 | 0.01% | 0.13 | 0.00% | 0.68 | 0.03% | 0 | 0.00% |
| **Beverage** | **337** | **1.92%** | **184** | **1.76%** | **17** | **0.97%** | **0.06** | **0.00%** | **23.88** | **0.39%** | **30.07** | **1.11%** | **1.83** | **0.17%** |
| Juice | 38 | 0.22% | 32 | 0.31% | 4 | 0.23% | 16.77 | 0.17% | 16.77 | 0.28% | 0 | 0.00% | 0 | 0.00% |
| Kombucha | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 8.75 | 0.09% | 2.92 | 0.05% | 5.84 | 0.22% | 0 | 0.00% |
| Smoothie | 178 | 1.01% | 45 | 0.43% | 9 | 0.51% | 14.59 | 0.15% | 3.6 | 0.06% | 9.22 | 0.34% | 1.77 | 0.17% |
| Chocolate | 5 | 0.03% | 3 | 0.03% | 0 | 0.00% | 0.89 | 0.01% | 0.01 | 0.00% | 0.86 | 0.03% | 0.02 | 0.00% |
| Coffee | 111 | 0.63% | 99 | 0.95% | 4 | 0.23% | 14.73 | 0.15% | 0.58 | 0.01% | 14.12 | 0.52% | 0.04 | 0.00% |
| Tea | 5 | 0.03% | 5 | 0.05% | 0 | 0.00% | 0.04 | 0.00% | 0 | 0.00% | 0.04 | 0.00% | 0 | 0.00% |
| **Pseudo meat** | **462** | **2.63%** | **155** | **1.48%** | **16** | **0.92%** | **0** | **0.00%** | **0** | **0.00%** | **0** | **0.00%** | **1.51** | **0.14%** |
| **Snack** | **1353** | **7.69%** | **796** | **7.60%** | **80** | **4.58%** | **390** | **4.02%** | **216.5** | **3.55%** | **121.34** | **4.47%** | **55.7** | **5.30%** |
| Confectionery | 71 | 0.40% | 50 | 0.48% | 3 | 0.17% | 0 | 0.00% | 0.15 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Snack Bars | 256 | 1.46% | 176 | 1.68% | 10 | 0.57% | 10 | 0.10% | 7.35 | 0.12% | 0.04 | 0.00% | 0 | 0.00% |
| Popcorn | 77 | 0.44% | 72 | 0.69% | 11 | 0.63% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Ice cream | 90 | 0.51% | 40 | 0.38% | 5 | 0.29% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Dessert | 369 | 2.10% | 83 | 0.79% | 28 | 1.60% | 140 | 1.44% | 12.8 | 0.21% | 79.72 | 2.94% | 50.82 | 4.84% |
| Biscuits | 137 | 0.78% | 38 | 0.36% | 11 | 0.63% | 230 | 2.37% | 184.46 | 3.03% | 41.58 | 1.53% | 4.88 | 0.46% |
| Crisps and Chips | 353 | 2.01% | 337 | 3.22% | 12 | 0.69% | 11.73 | 0.12% | 11.73 | 0.19% | 0 | 0.00% | 0 | 0.00% |
| **Seaweeds** | **7** | **0.04%** | **2** | **0.02%** | **0** | **0.00%** | **4100** | **42.31%** | **4056.87** | **66.59%** | **42.95** | **1.58%** | **0** | **0.00%** |
| **Dish** | **4640** | **26.37%** | **1967** | **18.79%** | **313** | **17.91%** | **1820** | **18.78%** | **1252.96** | **20.57%** | **275.5** | **10.15%** | **288.87** | **27.49%** |
| Tofu | 1786 | 10.15% | 1035 | 9.89% | 166 | 9.50% | 120 | 1.24% | 26.35 | 0.43% | 79.43 | 2.93% | 15.16 | 1.44% |

| Food source | Total PUFA | | LA | | ALA | | VLC_N3 | | EPA | | DPA | | DHA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Curry | 211 | 1.20% | 58 | 0.55% | 6 | 0.34% | 98.22 | 1.01% | 20.93 | 0.34% | 27.63 | 1.02% | 49.67 | 4.73% |
| Soup | 227 | 1.29% | 66 | 0.63% | 12 | 0.69% | 402.36 | 4.15% | 184.32 | 3.03% | 55.97 | 2.06% | 162.07 | 15.43% |
| Fried | 380 | 2.16% | 270 | 2.58% | 51 | 2.92% | 37.89 | 0.39% | 21.58 | 0.35% | 1.86 | 0.07% | 0 | 0.00% |
| Sushi | 58 | 0.33% | 38 | 0.36% | 4 | 0.23% | 957.44 | 9.88% | 950.22 | 15.60% | 7.22 | 0.27% | 0 | 0.00% |
| Dumplings | 95 | 0.54% | 81 | 0.77% | 4 | 0.23% | 67.68 | 0.70% | 0 | 0.00% | 26.05 | 0.96% | 41.63 | 3.96% |
| Nachos and tacos | 98 | 0.56% | 7 | 0.07% | 4 | 0.23% | 4.7 | 0.05% | 0 | 0.00% | 0 | 0.00% | 4.7 | 0.45% |
| Mix dish | 574 | 3.26% | 127 | 1.21% | 19 | 1.09% | 17.96 | 0.19% | 2.57 | 0.04% | 12.55 | 0.46% | 3.7 | 0.35% |
| Pasta and Noodles | 674 | 3.83% | 121 | 1.16% | 23 | 1.32% | 34.2 | 0.35% | 19.24 | 0.32% | 3.72 | 0.14% | 11.24 | 1.07% |
| Burger and Buns | 236 | 1.34% | 65 | 0.62% | 7 | 0.40% | 13.84 | 0.14% | 3.82 | 0.06% | 0.01 | 0.00% | 0.7 | 0.07% |
| Pizza and Pie | 151 | 0.86% | 31 | 0.30% | 4 | 0.23% | 61.23 | 0.63% | 23.93 | 0.39% | 37.31 | 1.37% | 0 | 0.00% |
| **Grand Total** | | **17594** | | **10469** | **1748** | | **9690** | | **6091.92** | | **2714.31** | | **1050.61** | |

* 1. Total intakes do not include contributions from PUFA supplements.

2. Portion (%) of total intake and grams (micrograms) per day of each PUFA contributed each food is presented as the mean of the whole population sample (n=193).

3. Abbreviation: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; VLCN3, DPA+DHA+EPA

### 3.4.6 Supplements of n-3 Polyunsaturated Fatty Acid

Although 193 participants completed four days food record, only 17 reported that they included n-3 supplements in their daily diet. The 4-day food record was the only place researchers asked about participants' n-3 supplements consumption. These supplements were not included in the dietary analysis. Table 3.5 summarises several of the n-3 supplements mentioned by these participants and their specific PUFA content. According to Table 3.5, the participants used various n-3 supplements, showing the diversity of different brands and formulations.

**Table 3.5 n-3 supplements detail**

| | Supplement name | n-3 (mg) | DHA (mg) | EPA (mg) | ALA (mg) | n-6 (mg) | LA (mg) |
|---|---|---|---|---|---|---|---|
| Omega 3's (ALA, EPA, DHA) | Lifestream vegan omega 3 | | 250 | 125 | | | |
| | Wagner Vegan Omega 3-6-9 Plus DHA | 250 | 80 | | 170 | | 40.8 |
| | Healthy Care Pure Vegan Omega 3-6-9 | | 200 | | | | |
| | Clinicians Vegan Omega-3 Algae Oil | 550 | 300 | 150 | | | |
| | DEVA Vegan Omega-3 | | 180-252 | 90-126 | | | |
| | The Good Vitamin Co Good Omega Vegan Friendly | 100 | | | | 19 | |
| | Good Health Flaxomega Organic Flax Oil | | | | 84 | | 22 |
| | Naturitas Vegan Omega 3 | | 250 | 4 | | | |
| | Naturelo Vegan DHA Supplement with Omega-3 from Algae | | 800 | | | | |

Abbreviation: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; VLCN3, DPA+DHA+EPA

## 3.5 Discussion

### 3.5.1 Overall Findings

The present study showed that New Zealand vegans mostly met the recommended LA and ALA intakes. Nevertheless, the intake of long-chain n-3 PUFA (e.g., EPA, DPA, and DHA) was below current guidelines. Nuts and seeds, tofu, imitation dairy products, and fats and oils were the primary sources of PUFA for vegans. While participants could fulfil their LA and ALA requirements through their dietary intake, due to the limited number of food sources intakes of EPA and DHA were insufficient and only a small number of participants utilised n-3 supplements.

### 3.5.2 Characteristics of the Participants

Participants in this study included 212 vegans from Auckland, New Zealand. Of these participants, 61% had been adhering to a vegan diet for over five years. There were 73% of females and 84.4% of participants of European ethnicity. In addition, the participants were generally well-educated. 68.9% of the participants had a bachelor's degree or higher. These data reflected the characteristics of the vegan population to some extent.

As a non-dietary factor, sex also plays a role in converting ALA to DHA/EPA (Alessandri et al., 2012). Studies demonstrated that females are more likely to convert ALA to DHA than males, primarily attributed to oestradiol’s positive role in DHA synthesis (Burdge et al., 2002). In contrast, males have significantly lower ALA conversion (Alessandri et al., 2012; Burdge et al., 2002; Burns-Whitmore et al., 2019). The NHMRC (2006) and the IOM (2002) recommended that men consume more ALA to compensate for the effects of sex differences on ALA conversion rates. These sex differences emphasise the need to consider sex-specificity when developing dietary guidelines, particularly for nutrients like omega-3 fatty acids significantly influenced by physiological differences. Therefore, future studies should endeavour to recruit a more diverse and representative sample of vegans from all regions of New Zealand, with balanced sex ratios, to gain a more comprehensive understanding of the dietary intake and nutritional status of vegans of different sexes, which could provide the basis for more targeted dietary recommendations.

### 3.5.3 Dietary Intake of n-3 LCPUFAs

This study focused on assessing the polyunsaturated fatty acid intake status and major food sources of vegans by collecting and analysing participants' 4-day Food Records results. There were 212 vegan participants, of which 193 completed and recorded their detailed 4-day dietary intake.

Overall, the participants in the study had dietary intakes of LA and ALA which aligned with NHMRC recommendations. The median LA intake for male participants was 14.42 g/d, higher than the NHMRC recommendation of 13 g/d, and for female participants was 12.05 g/d, higher than the recommendation of 8 g/d. Most participants met the NHMRC recommended intake of LA and ALA, 59.6% and 76.9% of males and 77.3% and 85.1% of females. Although most participants met the recommended intake of LA and ALA, there were significant shortfalls in the intake of EPA, DPA and DHA, which accounted for only 0.03%, 0.02% and 0.01% of the total PUFA intake, respectively. Only 3.5% of female participants achieved the NHMRC recommended AI for n-3 long-chain PUFA, indicating that most participants had inadequate intake of n-3 LCPUFAs.

The dietary intake of n-3 LCPUFAs in the participants was slightly different from the results in other studies. Vegan participants in many studies have higher LA/ALA dietary intake ratios than the results of this current study (Welch et al., 2010; Elorinne et al., 2016; Menzel et al., 2022; Pinto et al., 2017b). For example, the mean daily LA intake of the vegan population in Welch et al.'s (2010) study was 12.79 ± 10.80 g/d, and ALA intake was 1.02 ± 0.71 g/d. The LA/ALA ratio in our study was approximately 6:1, which is much lower than the ratio in vegan EPIC-Norfolk Study, 12.5:1 (Welch et al., 2010). The difference with the present study is that Welch et al. (2010) used a 24-hour recall record for one day and a 6-day food diary to record dietary intake data. The use of different methods and instruments for recording dietary intake, such as the food frequency questionnaire (FFQ), 24-hour recall and food diary, was also mentioned in other studies. Meanwhile, many food products available in the New Zealand market do not have detailed fatty acid content in their Nutrition Information Panel, and the dietary intake results obtained in this study for vegans may not be absolute. These studies all involved omnivores as a control group and found that vegans had lower intakes of n-3 LCPUFA than omnivores. Suppose future studies are set up and compared with an omnivore control group. In that case, it may be more intuitive to consider the differences in dietary intake of fatty acids between vegans and omnivores.

In addition, more female vegans than men achieved NHMRC-recommended AI intake for total n-3 LCPUFA (EPA+DPA+DHA) dietary intake in this study. It suggests that females participating in this study may have better n-3 LCPUFA dietary intakes outcomes than males. The similarity with the results of the present study is Welch et al., (2010) also mentioned higher n-3 LCPUFA dietary intake in females.

### 3.5.4 Current Dietary Intake Recommendations for n-3 PUFAs

The findings of this study are consistent with many other international studies of vegan diets, in which vegans have a higher risk of deficiency in n-3 LCPUFAs (Chamorro et al., 2020; Elorinne et al., 2016; Kornsteiner et al., 2008; Mann et al., 2006b; Menzel et al., 2022). Globally dietary intake recommendations for n-3 and n-6 polyunsaturated fatty acids (PUFAs)

are proposed by several health organisations. Most guidelines focus primarily on LA and ALA, with only a few providing specific dietary intake recommendations for the individual LCPUFA (EHA, DPA and DHA). For example, NHMRC (2006) only gives an AI and UL for Total LC n-3 but does not provide specific EHA, DHA and DPA recommendations. In New Zealand, many foods are not analysed for individual n-3 LCPUFA (EHA, DPA, DHA) because they contain minimal or no n-3 LCPUFA. Despite many studies demonstrating the health benefits of adequate intake of EHA, DPA and DHA, Recommended Dietary Intakes (RDIs) for individual n-3 LCPUFA (EHA, DPA, DHA) have not been established in New Zealand at this time due to a lack of conclusive evidence. This lack of specific guidance highlights the need for further research to ensure that vegans receive adequate n-3 LCPUFA.

### 3.5.5 Main Food Sources of PUFAs

Analysis of the 4-day Food Record provided by the participants, showed that nuts and seeds were the main sources of total PUFA (12.11%), LA (15.61%), ALA (23.96%) and DPA (69.51%). Tofu and plant-based dairy alternatives also contributed 10.15% and 8.56% of total PUFA intake, respectively, and accounted for a significant proportion of LA and ALA intake. Fats and oils accounted for 6.80% of total PUFA and contributed mainly to LA. Bread and biscuits accounted for 11.06% of total PUFA and 13.29% of DHA intake. Vegetables contributed the most to EPA intake at 37.98 per cent. Other important sources included savoury snacks and sweet snacks, which contributed 2.44% and 2.30% to EPA intake, respectively. Fruits also contributed to EPA (1.14%) and DPA (4.58%) intake, while cereals were the primary source of DHA at 22.61%. These data suggest that participants' PUFA intake relied heavily on nuts and seeds, tofu, plant-based dairy alternatives, fats and oils, and bread and biscuits, with a significant role in the intake of specific fatty acids such as ALA and DPA. The main n-6 and n- 3 food sources of the vegans in this study were consistent with the results in other vegan studies (Burns-Whitmore et al., 2019; Elorinne et al., 2016; Welch et al., 2010).

### 3.5.6 Current Consumption of n-3 LCPUFAs Supplements

There was limited evidence collected in this study of the consumption of n-3 LCPUFAs supplements from vegans. Out of the 193 individuals who completed the 4-day food record, only 17 individuals consumed n-3 supplements. The quantity of DHA and EPA in the supplements differed depending on the brand and formulation. For instance, Lifestream Vegan Omega 3 includes 250 mg of DHA and 125 mg of EPA, whereas Naturitas Vegan Omega 3 contains 800 mg of DHA. Several n-3 supplements can be found in the market. Consuming n- 3 fatty acids through these supplements can assist in addressing potential shortages in the diet, particularly for vegans. Nevertheless, only a few participants included these supplements in their diets, showing that most vegans do not utilise supplements to fulfil their n-3 fatty acid requirements. The potential positive impact of n-3 LCPUFA

supplementation on improving the n-3 LCPUFA status of vegans is a potential strategy for addressing the results of this study. Dams et al. (2020) showed that appropriate supplementation with vegan n-3 supplements positively affected blood n-3 fatty acid concentrations in vegans after just eight weeks. A study by Sarter et al. (2015) also proved that appropriate doses of vegan omega-3 supplementation could improve the n-3 LCPUFAs status of vegans.

### 3.5.7 Strengths and Weaknesses

The strengths of this study including a large sample size and a wide age range. In addition, this study assessed participants' intake of n-3 LCPUFAs and their main dietary sources, provided valuable data to support future studies and helped develop dietary guidelines specifically for New Zealand vegans.

There are also some limitations in this research. Participants were only based in Auckland, limiting this research's geographical representation of the entire New Zealand vegan population. The sample was predominantly female, resulting in insufficient data for male participants. Some food sources were not included in the database, and substituting similar foods may have reduced the accuracy of the analyses, potentially underestimating participants' n-3 LCPUFA intake. Furthermore, the study only examined four days of food records; future research needs to account for the long-term consumption of n-3 LCPUFAs. Lastly, the n-3 LCPUFA intake recommendations researchers used were not specifically focused on vegans, which may affect the applicability of the findings to this population.

## 3.6 Conclusion

This is the first study in New Zealand to examine the intake status and food sources of n-3 LCPUFAs in vegans. Although most participants consumed adequate LA and ALA, their n-3 LCPUFAs (EPA, DPA and DHA) were low. These results are concerning, and further in-depth studies may be needed to assess the risk of low n-3LCPUFAs (EPA, DPA and DHA) intake in New Zealand vegans. The study emphasises that people adhering to a vegan diet may need to consider their intake of n-3 LCPUFAs and that public health policies and nutritional guidance should encourage health maintenance by ensuring adequate DHA and EPA intake through diet and appropriate supplements.

# Chapter 4: Conclusions and Recommendations

## 4.1 Conclusions

The main objective of this study was to assess the dietary intake and main food sources of omega-3 polyunsaturated fatty acids (n-3 LCPUFAs) in New Zealand vegans. Firstly, this study found that the overall dietary intake of LA and ALA in New Zealand vegans was favourable. Most participants achieved the NHMRC Adequate Intake (AI) for LA and ALA, which suggested that these nutrients may not be of particular concern for vegans. Secondly, the results showed that 96.5% of New Zealand vegans' dietary intake of total n-3 LCPUFAs (EPA+DPA+DHA) was below the recommended amount, with only a small number of female vegans (n=5) meeting the AI. Fish and other deep-sea animal products are the main sources of DHA and EPA (Tur et al., 2012). Since vegans do not consume fish, their limited food sources of DHA and EPA contribute to their low n-3 LCPUFA intake (Chamorro et al., 2020; Menzel et al., 2022). Additionally, the study found that male New Zealand vegans consumed significantly more energy, total fat, polyunsaturated fatty acids, and LA per day than female vegans. However, there were no significant gender differences in the intake of n-3 LCPUFAs, ALA, EPA, DPA, and DHA.

The study also found the main food sources of LCPUFAs for New Zealand vegans could be divided into a number of specific food groups. Nuts and seeds were the primary sources of LA, ALA, and DPA. Tofu and pseudo-dairy products accounted for a large proportion of the total PUFA, LA, and ALA intake. Bread and crackers were the main sources of DHA, while vegetables and fruits were the main sources of EPA. Overall, the primary food sources of total n-3 LCPUFAs (EPA+DPA+DHA) for vegans were seaweeds (66.6% EPA), nuts and seeds (69.5% DPA), and cereals (51% DHA). However, these food sources have a limited impact on improving the n-3 LCPUFA intake status of vegans (Lane et al., 2022). Only 9% of participants in this study consumed n-3 LCPUFA supplements. Appropriate use of supplements may help vegans improve their dietary intake of n-3 LCPUFAs (Arterburn et al., 2007; Geppert et al., 2006). This is the first study to investigate the dietary n-3 LCPUFAs intake and main food sources of vegans in New Zealand. With the popularity of vegan diets in New Zealand and globally, this study of vegans is particularly well-timed. Most vegans consumed adequate amounts of LA and ALA, but their dietary intake of n-3 LCPUFAs was poor. Therefore, vegans should eat more EPA- and DHA-rich foods such as nuts, seeds, and deep-sea plants to supplement their n-3 LCPUFAs. The results of this study enrich the understanding of vegans in New Zealand and provide evidence for the future development of appropriate dietary guidelines for vegans. However, there are some limitations to this study. As most participants were female vegans in the Auckland region, the study's results may not apply to all groups of vegans. It is important to be cautious when using the results of this

study, especially for geographically and gender- diverse vegans.

## 4.2 Strengths and Limitations

This study has several significant strengths. Firstly, the large sample size spanned a wide age range of participants. There were 212 participants recruited for this study with a mean (SD) age of 39.43 (12.41) years. Secondly, the participants were highly motivated, ensuring the study's smooth running. Participants in this study were keen to complete the study and provide accurate data. There were 193 of the 212 participants in this study who submitted compliant and detailed 4-day food records, which provided reliable data for nutritional analyses. Finally, this study is the first to focus on a specific vegan population in New Zealand. It fills a research gap regarding the n-3 LCPUFA intake status of vegans and their food sources, which is important in relevance and application. The data analysis section in this study details the analyses of PUFA intake and their food sources in New Zealand vegans.

Evidence from this study may help vegans better understand their dietary intake of n-3 LCPUFAs and help them meet their nutritional needs through rational food choices and supplement use. In addition, the results of this study have important implications for the future development of dietary guidelines for New Zealand vegans. It could be a basis for public health organisations to provide more accurate nutritional guidance for vegans.There are some limitations to this study. Firstly, most participants were New Zealand European female vegans from Auckland, resulting in limited ethnic, geographic, and gender diversity. Additionally, this study did not assess possible misreporting by participants during the four-day food record process. Although detailed records were obtained for the 4-day food record, there is always the limitation of missing data, poor recall and misinterpretation of portion sizes, which can impact the quality of the dietary data.

Furthermore, the study used FoodWorks software to analyse participants' dietary intake. The database did not include some current vegan products and ingredients on the market. Researchers substituted similar foods for those not included in the database, which may have reduced the accuracy of the dietary n-3 LCPUFA intake analysis. Although the FoodWorks database provides complete values for most individual PUFAs, it does not report total n-6 and n-3 values, preventing the comparison of dietary intake ratios of n-6/n-3 LCPUFAs in vegans.

The study only examined participants' dietary intake over four days, without assessing their long-term n-3 LCPUFA intake. This limitation makes it difficult to determine the health effects of long-term dietary patterns and may not fully reflect the actual intake of n-3 LCPUFAs by vegans. The National Health and Medical Research Council (NHMRC) provided Adequate Intake (AI) values for adults on a normal diet, which have not been adjusted for vegan adults

and may not accurately represent the dietary nutritional requirements of vegans.

Finally, this study did not consider the use of supplements when assessing the dietary intake of n-3 LCPUFAs. Although a small number of participants reported using supplements, they were not included in the final analyses.

## 4.3 Recommendations

- Include a more diverse sample of vegans, incorporating participants from different ethnicities and ensuring a balanced gender representation. Analyse and understand how these factors influence n-3 LCPUFAs intake and overall dietary patterns.
- Conduct a nationally representative study to assess differences in dietary intake between vegan populations in different parts of New Zealand (including rural and urban areas, different regions, and the North and South Islands). This would help to increase the generalisability of the findings.
- Validate dietary assessment methods in a vegan population to determine the accuracy and reliability of dietary intake in vegan populations.
- Conduct longitudinal studies to assess long-term dietary intake of n-3 LCPUFAs and its impact on health outcomes such as cardiovascular health, cognitive function, and inflammatory markers.
- Update and expand the nutrient database used in the dietary analysis software to include the latest vegan products and ingredients, ensuring a more accurate assessment of individual n-3 LCPUFAs intake.
- Explore physiological and metabolic differences in the conversion of ALA to DHA and EPA in New Zealand male and female vegans, which may allow for the adjustment of dietary recommendations accordingly.
- Conduct detailed bioavailability analyses of the main food sources of n-3 LCPUFAs consumed by New Zealand vegans to provide evidence for future dietary recommendations to this population

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# Appendices:

## Appendix 1: Participant Information Sheet

### Participant Information Sheet

**Health and Vegan Diet**

A clinical investigation project included in Phase 2 of The Vegan Health Research Programme

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Lead Researcher: Professor Pamela von Hurst

Study Site: Human Nutrition Research Unit, Massey University, Albany

Contact phone number: 09 414 0800 ext 43657

Ethics committee ref.: 2022 EXP 12312

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You are invited to take part in a study investigating the impact of a vegan diet on your health. Whether or not you take part is your choice. If you want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

This form is 8 pages. Please make sure you have read and understood all the pages.

#### VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Participation in this study is completely voluntary. You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular questions
- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information on the understanding that your name will not be used
- Be given access to a summary of the study findings when it is concluded

Withdrawing from the study, should you choose to, will not result in any disadvantage to you.

#### WHAT IS THE PURPOSE OF THE STUDY?

Interest in the vegan lifestyle is growing, and NZ ranks the fifth most vegan country in the world. A vegan diet tends to have some health benefits, but at the same time it might be associated with nutrient deficiencies.

These deficiencies could have significant health consequences if they occur during critical period of life (for example, pregnancy or the rapid growth and developmental stages). Therefore, dietary guidelines stress that those who follow strict vegetarian or vegan diets may

need extra information and/or support to ensure that they meet their nutrient needs. Our search has not found any studies to date that have investigated nutritional status, nutrient/food intake, motivations and nutritional knowledge and their sources of NZ vegans.

The aims of this study are to investigate nutritional status, nutrient/food intake, reasons for becoming vegan, nutrition knowledge and sources of nutrition information, and gastrointestinal discomfort symptoms among NZ vegans.

## HOW IS THE STUDY DESIGNED?

This study will involve 220 men and women aged 18 years or older, who have been on a vegan diet for at least two years. Participants will take part in online or telephone screening to check eligibility. If eligible they will visit the Human Nutrition Unit at Massey University, once for approximately 90 minutes,

Participants will be required to have bone density, body composition, and blood pressure measurements, complete online questionnaires regarding health, demographics, lifestyle, physical activity, motivations for following a vegan diet, dietary intake, nutrition knowledge, and sources of nutrition knowledge, and complete a 4-day diet record. In addition, participants will be asked to provide a non-fasted blood sample.

## WHO CAN TAKE PART IN THE STUDY?

Men and women aged 18 years or older, who have been following a vegan diet for at least two years will be included in this study. Women who are pregnant or have any likelihood of being pregnant will be excluded from this study. Participants will complete a short screening questionnaire to ensure they meet inclusion criteria.

## WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

If you decide to take part in this study, after you have read and had time to consider the information in this information sheet, you will be required to complete the screening questionnaire. Screening involves answering a few inclusion criteria questions, this can be done at home either online or on the phone, and takes approximately five minutes. Your answers to this questionnaire will help us to see if you are eligible to take part in this study or not.

If you are eligible to take part in this study, you will be required to visit Human Nutrition Unit at Massey University in Albany on one occasion for data collection. Prior to your visit to Massey University, we will send you a consent form, some questionnaires that need to be completed online, and a diet diary. For the online questionnaires, we will ask you to:

- Complete demographic, health, lifestyle, and physical activity questionnaires.
- Complete a questionnaire to assess motivations for following a vegan diet
- Complete a questionnaire to assess dietary intake
- Complete a questionnaire to assess nutritional information and their sources
- Complete a questionnaire to assess gastrointestinal discomfort symptoms

For the diet diary, we request that for 4 days you record everything you eat and drink. Instructions will be provided in more detail at your visit.

A researcher will make an appointment with you at your convenience You will be required to not have caffeinated drinks and not exercise for 2hrs prior to the visit. This visit will take approximately 90 minutes and you will be reimbursed for your travel.

At this appointment you will first be asked to hand in the signed consent form for participating in the study and you will have the opportunity to ask any questions you may have about the study. During this visit, we will ask you to

- Have weight, height, and waist and hip circumferences measured by a trained researcher.
- Have bone density and body composition measured using dual-energy X-ray absorptiometry (DXA). This machine uses very low dose X-rays to measure the bone density of your hip and spine, and also measures your body composition (fat mass, lean mass, and bone mass of your body).
- Have blood pressure measured using electronic blood pressure monitor by a trained researcher
- Provide a small venous blood sample (about 20ml which is equivalent to 4 teaspoons). This will be taken by a qualified phlebotomist. It will be used to measure levels of various nutrients in your blood, such as iron and vitamin D.

## WHAT WILL HAPPEN TO MY BLOOD SAMPLES?

All samples will be labelled with the participant's unique identity code/number and not by the participant's name.

The blood samples will be stored in a minus 80 degree freezer until the study is completed after which time the biochemical analysis will be conducted. While waiting for data and bloods to be collected from all participants and analysed in one batch, samples will be kept in the freezer at the Nutrition laboratory at Massey University, Building 27, Oteha Rohe campus, Albany.

On completion of the study, samples will be sent to the Canterbury Health Labs to assess vitamins D, $B_{12}$, folate, iron, lipids, calcium and albumin.

One drop of whole blood sample will be analysed on site at Massey University to assess haemoglobin, and another drop will be applied to a special paper to be sent to CSIRO laboratory in Adelaide to assess polyunsaturated fatty acids.

Participants may ask to withdraw their samples at any time during the study up to the time the samples are analysed. The analysis results in the destruction of the sample.

There may be participants who identify as Māori and if specific concerns develop, the support of Dr Bevan Erueti (Taranaki, Te Ati Haunui-ā-Papārangi, Ngāti Tūwharetoa), Associate Dean Māori, will be afforded. Dr Erueti has expressed that he is happy to act in the capacity of advisor and if required will assist and facilitate the projects Māori agenda and ensure that relational aspects of trust and appreciation are upheld with Māori participants. We are also aware that a diversity of beliefs and cultural concerns regarding the removal, storage and transport of tissue samples and these should be discussed with your whānau (family) or take advisement from hapū and iwi leaders. Nonetheless, the right to decline or withdraw from the study can be done at any stage of the project.

## WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

The DXA has X-ray beams of different energies and, while no dose of radiation is harmless, this dose is very low and unlikely to cause harm. The total effective dose of radiation to which you will be exposed to is 10.8 microsieverts (μSv), which is much lower than the range normally used in medical diagnostics. To place this in perspective, the amount of radiation an individual would receive from flying in an aircraft to the United Kingdom equates to an effective dose about six times that received from the study. The effective dose received by the

participants from the study is also equivalent to about 2 days of background radiation to which all New Zealanders are exposed. This procedure is quick, non-invasive and completely painless. The room is private, and the staff are experienced and certified.

Some people may have a fear of having a blood sample taken or experience discomfort when blood samples are taken. Occasionally a slight bruising will result. The bruising usually disappears within a day or two. Blood samples will be taken by a trained phlebotomist. There may be social or cultural discomfort from having a blood sample, bone density, body composition, and blood pressure measurements taken, however, you will be treated with respect, and privacy will be ensured. We will explain all measurements being taken and ask for your permission prior to undertaking these measurements. You may also be accompanied by a support person if you wish. Every effort will be made to ensure your comfort and respect your participation.

## WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

- You will be contributing to a greater understanding of the health implications of a vegan diet.
- You will not be charged for any of the measurements conducted for the study
- You will be provided with your body composition results, blood test results and a nutrient analysis of your diet from your 4-day diet diary.
- You will get a summary of the study results.

## WILL ANY COSTS BE REIMBURSED?

Participants will not incur any costs as part of being involved in the study and will receive reimbursement for travel ($20 in vouchers).

## WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, you would be eligible to apply for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

## WHAT WILL HAPPEN TO MY INFORMATION?

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

Identifiable Information

Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). The following groups may have access to your identifiable information:

- Research staff (to complete study assessments)
- Government agencies, like HDEC, ACC and its representatives, if you make a compensation claim for study-related injury. Identifiable information is required in order to assess your claim.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researcher. Instead, you will be identified by a code. The researcher will keep a list linking your code with your name, so that you can be identified by your coded data if needed.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Anonymised Information

The lead researcher may remove the code from your de-identified information – this is called 'anonymisation'. This makes it very difficult (but not impossible) to identify the information that belongs to you. The researcher may share this anonymised information with other researchers on request for the purpose of accumulating data from individual studies. The anonymous/anonymised data is unable to be accessed, corrected, or withdrawn; and return of individual results will not be possible.

Future Research Using Your Information

If you agree, your fully anonymous/anonymised information may be used for future research related to veganism. This is optional and you could still participate in the present study if you do not agree.

This future research may be conducted overseas. You will not be told when future research is undertaken using your information. Your information may be shared widely with other researchers. Your information may also be added to information from other studies, to form much larger sets of data.

You will not get reports or other information about any future research that is done using your information.

Your information may be used indefinitely for future research unless you withdraw your consent. However, it may be extremely difficult or impossible to access your information, or withdraw consent for its use, once your information has been shared for future research.

Security and Storage of Your Information

Your identifiable information is held at Massey University during the study. After the study it is transferred to a secure archiving site and stored for at least 10 years, then destroyed. Your coded information will be entered into electronic case report forms. Coded study information will be kept in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

The linked data in this study will be destroyed at the end of the study.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your

information (e.g. making it harder for you to get or keep a job or health insurance) is currently very small but may increase in the future as people find new ways of tracing information.

Rights to Access Your Information

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.
Please ask if you would like to access the results of your scan (bone density and body composition) during the study. You can't access other study-specific information (e.g. diet analysis and blood test results) during the study, because these data will be analysed when the data from all participants are collected and the study is over.

If you have any questions about the collection and use of information about you, you should ask researcher.

Rights to Withdraw Your Information

You may withdraw your consent for the collection and use of your information at any time, by informing the study researchers.

If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.

Information collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

## WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

If you wish to withdraw from the study, please inform one of the research team. Information and data collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

The data will be used for the purposes of this study, and fully anonymised, selected outcomes may be shared with other researchers on request for the purpose of accumulating data from individual studies. Only investigators and administrators of the study will have access to personal information, and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number. Results of this project may be published or presented at conferences or seminars. No individuals will be able to be identified.

At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the project depend will be retained in secure storage for 10 years, after which it will be destroyed.

All participants will have access to a summary of the project findings when the study is completed.

## CAN I FIND OUT THE RESULTS OF THE STUDY?

All participants will have access to a summary of the project findings when it is completed. However, findings of any future research conducted using fully anonymised data collected in this project will not be made available to participants.

## WHO IS FUNDING THE STUDY?

This study is funded by the Lottery Health Project Grant.

Participants will not incur any costs for taking part in the study and will be reimbursed for travel.

## WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that studies meet established ethical standards. The Central Health and Disability Ethics Committee has approved this study.

## WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

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

Dr. Hajar Mazahery, study manager
Email: h.mazahery@massey.ac.nz

Rebecca Paul, research assistant
Phone: 022 1294112
Email: veganstudy@massey.ac.nz

The other members of the research team are: Professor Pamela von Hurst, Associate Professor Cathryn Conlon, Associate Professor Kathryn Beck, and Dr. Rachel Batty (College of Health, Massey University).

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@advocacy.org.nz
Website: https://www.advocacy.org.nz/

For Maori health support please contact:

Dr Bevan Erueti, Taranaki, Te Ati Haunui-ā-Papārangi, Ngāti Tūwharetoa, Associate Dean Māori
Phone: 06 356 9099 Ext 83087
Email: B.Erueti@massey.ac.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHIC
Email: hdecs@health.govt.nz

## Appendix 2: Screening Questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

# Health Implications of a Vegan Diet

### Screening Questionnaire

1. Are you a vegan?
   - Yes
   - No (exclude)

2. How long have you been following a vegan diet?
   - 2 years or more
   - Less than 2 years (exclude)

3. What is your date of birth (dd/mm/yyyy)?

   ..... (exclude if <18 years old)

4. What's your gender?
   - Male
   - Female
   - Gender diverse

5. Are you currently pregnant or have a likelihood of being pregnant?
   - Yes (exclude)
   - No
   - Not applicable

# Appendix 3: Health and Demographic Information

**Demographics (7 questions)**

48. When were you born?

_____ Day (DD) _____ Month (MM) _____ Year (YYYY)

49. What is your gender?

[ ] Female
[ ] Male
[ ] Gender diverse
[ ] Other (Please state)

50. Which ethnic group do you belong to? Tick whichever applies to you (you may check [x] more than one box)

[ ] New Zealand European
[ ] Māori
[ ] Samoan
[ ] Cook Islands Māori
[ ] Tongan
[ ] Niuean
[ ] Chinese
[ ] Indian
[ ] Others, eg DUTCH, JAPANESE, TOKELAUAN.
Please state below.
a) _____
b) _____

51. What is your HIGHEST level of EDUCATION?

[ ] Lower than high school
[ ] High school
[ ] Diploma/certificate
[ ] Bachelor's degree
[ ] Master's degree
[ ] Doctorate or PhD

52. Do you have tertiary education in the following fields? (you can choose more than one answer)

[ ] Medicine
[ ] Nutrition/Dietetics
[ ] Nursing
[ ] Midwifery
[ ] Other health related fields (Please specify)
[ ] Others (Please specify)
[ ] Not applicable

53. What is your current employment status?

[ ] Full time
[ ] Part time
[ ] Volunteer
[ ] Seeking opportunities currently
[ ] Retired
[ ] Other (e.g., caregiver, studying, homemaker). Please describe.

54. What is your marital status?

[ ] Married / cohabiting / civil union / de facto
[ ] Divorced / Separated
[ ] Widowed
[ ] Single
[ ] Other (please describe)

55. How many children have you given birth to? (If female)

[ ] No children
[ ] 1 child
[ ] 2 children
[ ] 3 children
[ ] 4 children
[ ] 5 or more children

## Appendix 4: 4 Day Food Record Template

StudyID: _____

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

# Health and Vegan Diet

### 4 Day Food Record

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

*If you have any questions, please contact Rebecca Paul on 022 1294112 (Email: veganstudy@massey.ac.nz)*

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

*Please bring the food diary with you when you come in for assessment at Massey University.*

StudyID: _____

# 4 day food diary - what to do?

- Record all of the food that you eat and drink on the following dates.
- **Please complete the diary on consecutive days for 1 weekend day and 3 week days at your convenience. For example, Sunday, Monday, Tuesday and Wednesday OR Wednesday, Thursday, Friday and Saturday.**
- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.
- Include all meals, snacks, and drinks, even tap water.
- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.
- Write down any information that might indicate size or weight of the food to identify the portion size eaten.
- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.
- Use as many pages of the booklet as you need.
- You can also save any packets such as muesli bar wrappers and bring them in with your food diary

## Describing Food and Drink

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

| General description | Food record description |
|---|---|
| Breakfast example – cereal, milk, sugar | 2 Weetbix (Sanitarium)<br>1 cup So Good unsweetened almond milk<br>1 tsp Chelsea white sugar |
| Lunch – Meat Free Bacon Style Rashers sandwich and home-made fries | 2 slices of wholegrain bread (Vogels)<br>2 slices Vegie Delights Meat Free Bacon Style Rashers<br>25g zenzo Dairy Free Vegan Cheddar Cheese Alternative<br>2 tsp Tablelands Dairy Free Buttery Spread<br>½ cup fries (home-made, deep fried in Pam's sunflower oil) |

StudyID: _____

| | |
|---|---|
| | ½ Tbs vegan aioli (Heinz Mayonnaise Vegan Aioli)<br>Water 1 cup to drink |
| Dinner – Vegan lentils spaghetti bolognese | ½ cup lentil sauce (see attached recipe)<br>1 cup spaghetti pasta (Homebrand) |
| Snacks | Tam & Luke Snack Ball Salted Caramel (2 balls, 28g)<br>1 small banana<br>2 Salada crackers with 1 tsp peanut butter<br>20g Doritos Spicy Sweet Chili Flavored Tortilla Chips |

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

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

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

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

- Please specify the **actual amount of food eaten** (eg. for leftovers, foods where there is waste)

| General description | Food record description |
|---|---|
| Apple | 1 x 120g Granny Smith Apple (peeled, core not eaten – core equated to ¼ of the apple) |

StudyID: _____

| | |
|---|---|
| Fried chicken alternative strips | 100g chicken alternative strips (100g includes batter); fried in 3 Tbsp Nuttelex buttery margarine |

| General description | Food record description |
|---|---|
| Milo | I x cup Milo made with plant based Milo powder and 150mls So Good unsweetened almond milk, 100 ml hot water. No sugar |

- **Record recipes** of home prepared dishes where possible and the proportion of the dish you ate. There are blank pages for you to add recipes or additional information.

**Recording the amounts of food you eat**

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

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

| General description | Food record description |
|---|---|
| Cheese alternatives | 1 heaped tablespoon of grated dairy free cheddar cheese<br>1 slice dairy free cheddar cheese (8.5 x 2.5 x 2mm) |

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|---|---|
| | 1 cube dairy free cheddar cheese, match box size |

- If you go out for meals, describe the food eaten in as much detail as possible.

- ***Please try to eat as normally as possible – e.g., Don't adjust what you normally eat just because you are keeping a diet record and be honest! This record will give us important information about your diet, and help us identify any possible deficiencies which we can then help you correct.***

Example day

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

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|---|---|---|
| 6.30pm | Vegan banana cake with chocolate icing (homemade, recipe attached) | 1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing |
| " " | Tip Top Crave dairy free salted caramel fudge frozen dessert | 1/2cup (g) (125g) |
| | | |

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**Date_____ DAY 1**

| Time food was eaten | Complete description of food (food and beverage name, brand, variety, preparation method) | Amount consumed |
|---|---|---|
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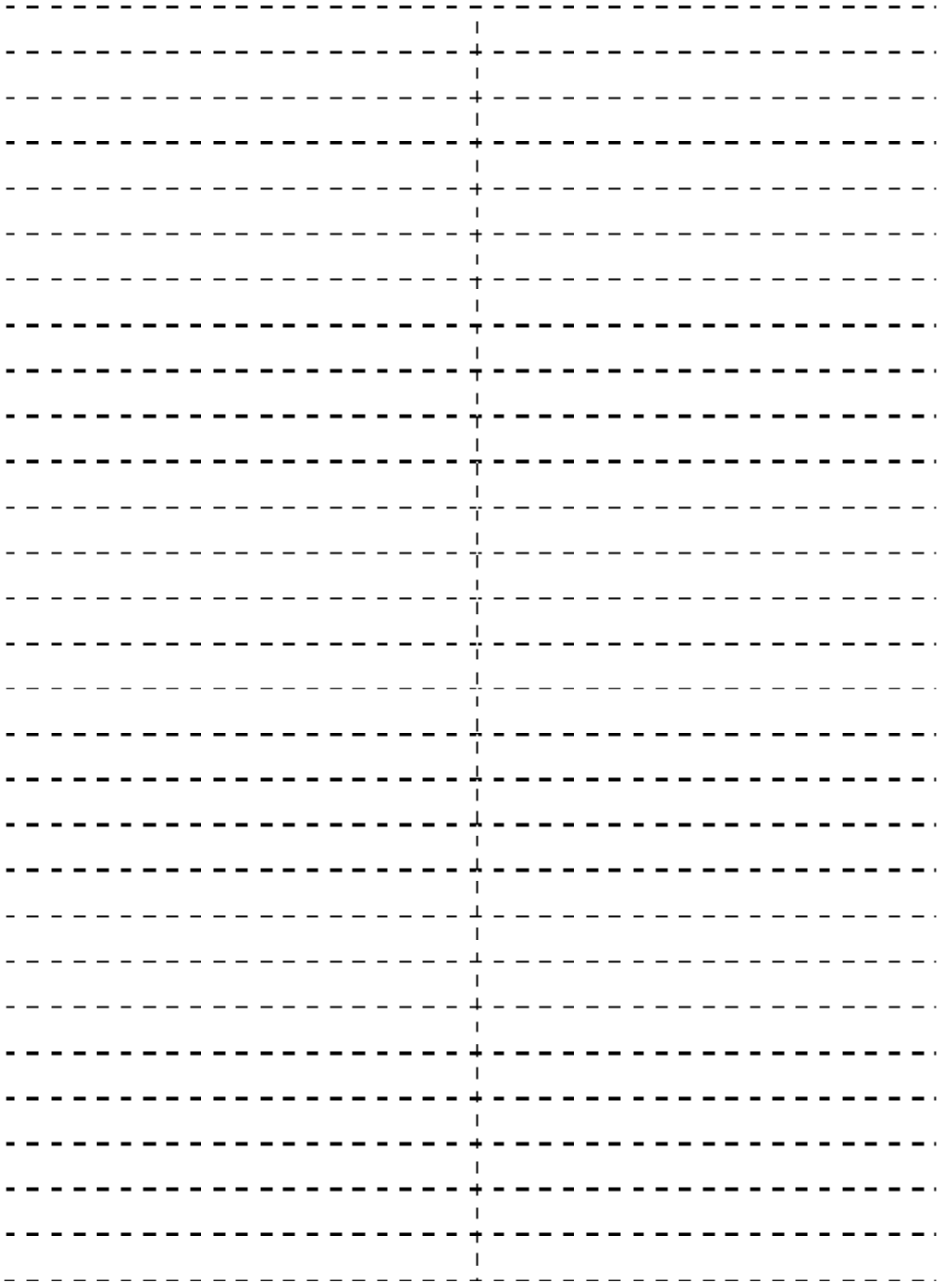
**Recipes (Day 1)**



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**Date_____ DAY 2**

| Time food was eaten | Complete description of food (food and beverage name, brand, variety, preparation method) | Amount consumed |
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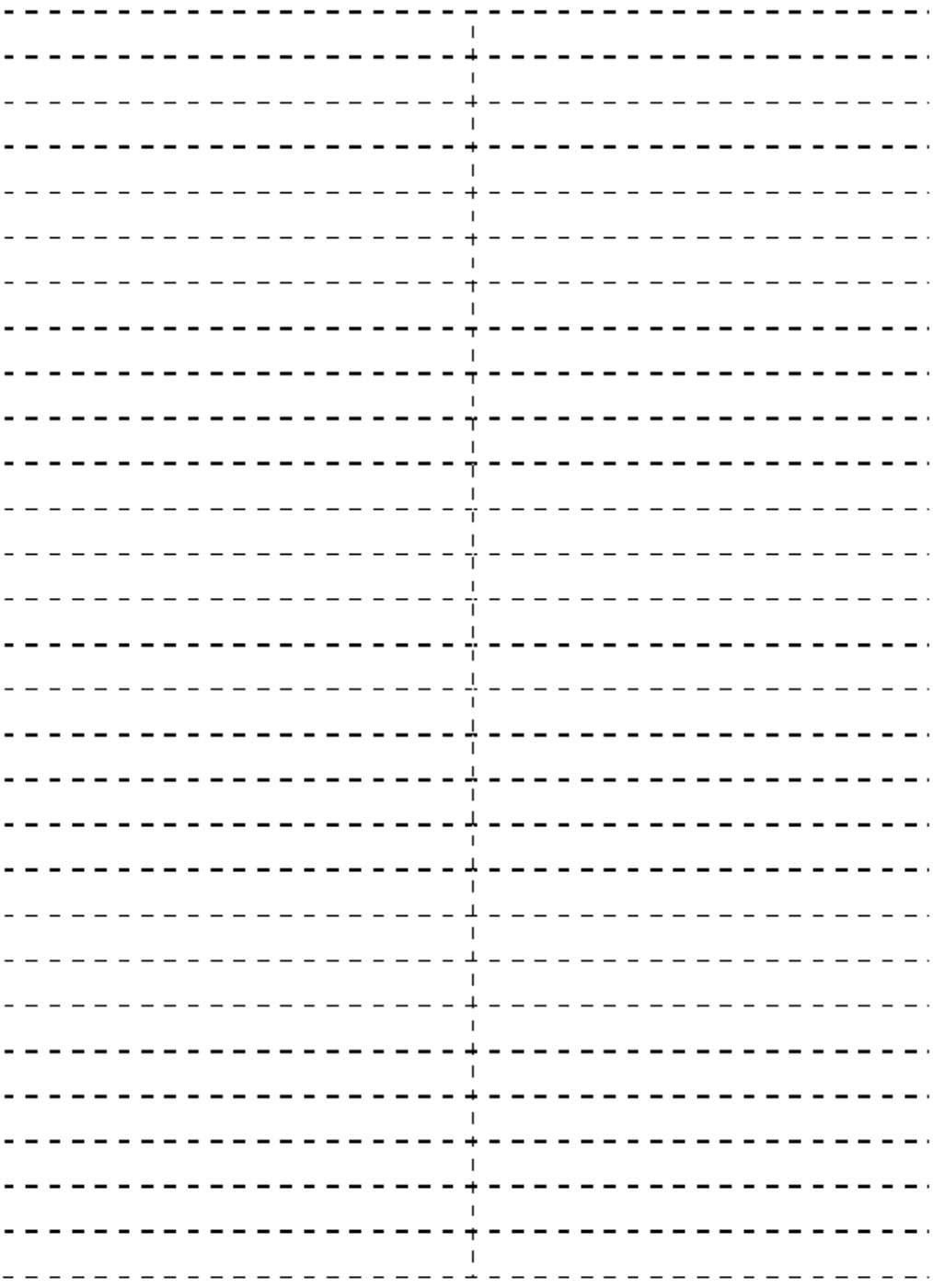
**Recipes (Day 2)**



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**Date_____ DAY 3**

| Time food was eaten | Complete description of food (food and beverage name, brand, variety, preparation method) | Amount consumed |
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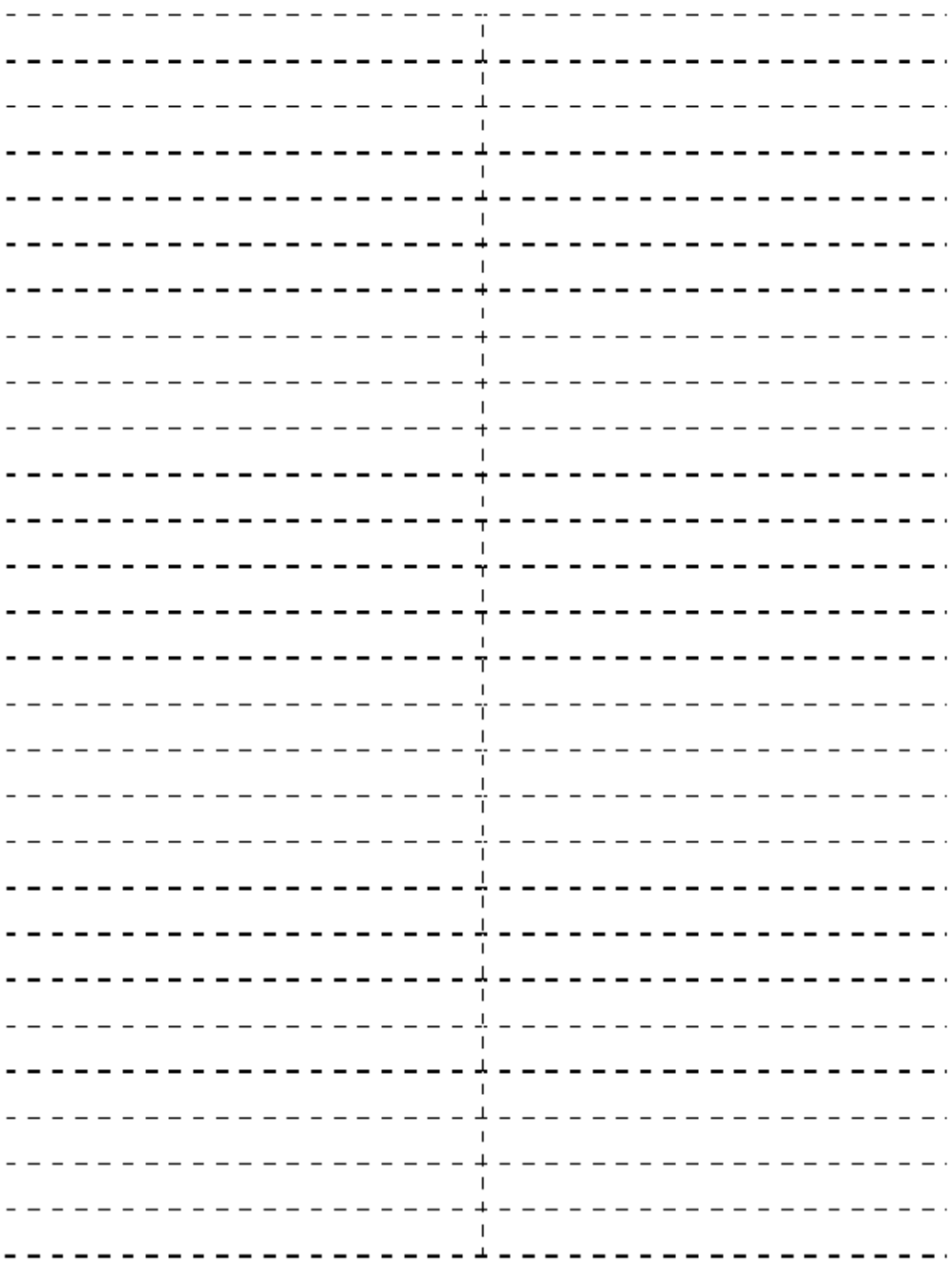
**Recipes (Day 3)**



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**Date_____ DAY 4**

| Time food was eaten | Complete description of food (food and beverage name, brand, variety, preparation method) | Amount consumed |
|---|---|---|
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**Recipes (Day 4)**