Determinants of Iron Status in Vegans Living in Auckland, New Zealand

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Abstract

Background: A vegan diet has many health benefits; however, certain nutrients are not available in sufficient quantities or are less bioavailable in plant food. One example is iron, presenting a risk for iron deficiency (ID). Research on iron intake and status in vegans is limited and has not been explored in a NZ vegan sample population.

Objectives: To investigate iron intake and status in a NZ vegan sample population. As well as exploring risk for ID and potential risk factors for iron depletion in this sample population.

Methods: Vegans living in Auckland, NZ were recruited. Nutrient intake was gathered through participant-completed four-day food records (4D-FR). Biomarkers of iron status were measured including Serum ferritin (SF), haemoglobin (Hb), serum iron, iron-binding capacity (TIBC) and transferrin saturation (TSAT). Health, demographic and lifestyle factors were assessed through questionnaires. Participants were grouped as ID (SF <30µg/L) and iron sufficient (SF ≥30µg/L) and the differences between groups were assessed.

Results: Vegan males and females (n=212) aged 19-75 years participated. Mean iron intake was above estimated average requirements (EAR) and recommended dietary intake (RDI) for males and above EAR for females. The prevalence of ID was 47.3% overall. Significantly higher rates of ID were found in females (F) (58.7%) compared to males (M) (15.4%) ($p \leq 0.001$). In all participants, being female ($p \leq 0.001$), younger age ($p \leq 0.001$), a previous diagnosis of iron deficiency ($p \leq 0.001$), and blood donation within the last six months ($p = 0.004$) were potential risk factors of ID. In females, being younger ($p \leq 0.001$), blood donation within the last six months ($p = 0.025$), and still menstruating ($p = 0.010$) were significant potential risk factors of ID. In males, energy ($p \leq 0.001$), protein ($p = 0.004$), dietary fibre ($p \leq 0.001$), iron ($p = 0.001$), calcium ($p = 0.003$) and vitamin C ($p = 0.006$) intake was significantly higher in ID (n=6) compared with iron sufficient (n=41) males. No significant differences in dietary intake were observed between ID and iron sufficient females.

Conclusion: Nearly half of the vegan sample was ID, with over half of vegan females ID. Iron deficiency was most prevalent in females, younger individuals, those previously diagnosed with ID and those that had donated blood within the last six months. Dietary intake was only found to be associated with ID in males and not females. This study has provided novel insights on the risk of ID for vegans living in New Zealand.

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List of Abbreviations

24HR	24-hour diet recall
ALA	Alpha linolenic acid
ATP	Adenosine triphosphate
BMI	Body mass index
BMP-SMAD	Bone morphogenic-SMAD
CRP	C-reactive protein
CVD	Cardiovascular disease
DALY	Disability adjusted life years
DHA	Docosahexaenoic acid
DMT1	Divalent metal transporter 1
DNA	Deoxyribose nucleic acid
FR	Food record
FFQ	Food frequency questionnaire
EAR	Estimated average requirement
EPA	Eicosapentaenoic acid
ETC	Electron transport chain
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
Fe-S	Iron sulphur
FFQ	Food frequency questionnaire
FPN	Ferroportin
Hb	Haemoglobin
HCT	Haem carrier protein
HMB	Heavy menstrual bleeding
HME-OMN	High meat eater
HO-1	Haem oxygenase-1
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDNA	Iron deficiency and no anaemia
LDL	Low density lipoprotein
LOV	Lacto-ovo vegetarian
MME-OMN	Moderate meat eater
MSc	Master of Science
NIP	Nutrition information panel
NNS	New Zealand nutrition survey
OW	Old women
PES	Pescatarian
PF	Plasma ferritin
RBC	Red blood cell
ROS	Reactive oxygen species
SD	Standard deviation
SF	Serum ferritin
STfR	Soluble transferrin receptor
S-V	Semi-vegetarian
T2D	Type 2 diabetes
Tf	Transferrin
TG	Triglycerides
TSAT	Transferrin saturation
VG	Vegan
WHO	World Health Organisation
YW	Young women

Chapter 1: Introduction

1.0 Introduction

In 2018 it was estimated that 1.1% of the New Zealander (NZ) population were following a vegan diet (Milfont, Satherley, Osborne, Wilson, & Sibley, 2021). Those adopting a vegan lifestyle are often motivated by concerns for animal welfare, the environment, and a desire to improve personal health (Janssen, Busch, Rödiger, & Hamm, 2016).

Following a vegan diet may lead to better health outcomes and a lower risk of developing certain conditions compared to other dietary patterns. Compared to omnivores, vegans tend to have a lower body mass index (BMI), waist circumference, low density lipoprotein (LDL), cholesterol concentrations, triglycerides (TG), fasting blood glucose and systolic and diastolic blood pressure (Benatar & Stewart, 2018). Along with a favourable metabolic status, studies have also found decreased incidence and mortality from ischemic heart disease and incidence of type 2 diabetes mellitus (T2DM) in vegans (Dinu, Abbate, Gensini, Casini, & Sofi, 2017; Kim et al., 2019). The vegan diet may also significantly reduce the incidence risk of overall incidence of cancer (Dinu et al., 2017; Tantamango-Bartley, Jaceldo-Siegl, Fan, & Fraser, 2013).

Research has identified nutrients in the vegan diet which may confer these health benefits. A vegan diet excludes all flesh foods as well as animal products, such as eggs, milk, and cheese (Tuso, Ismail, Ha, & Bartolotto, 2013). Omitting flesh and animal products from the diet removes a significant source of fat, namely saturated fatty acids (SFA). Intake of SFA in vegans is significantly lower than omnivores (Bakaloudi et al., 2021). Replacing saturated fats with other unsaturated fats and/or high-quality carbohydrates may decrease the incidence of coronary heart disease (Li et al., 2015). The vegan dietary pattern is also often lower in total energy, which is likely responsible for the lower BMI found in vegans (Benatar & Stewart, 2018). Vegans also have a higher fibre intake compared to meat-eaters (Davey et al., 2003; Neufingerl & Eilander, 2021). Dietary fibre is associated with metabolic, cardiovascular and bowel health (Barber, Kabisch, Pfeiffer, & Weickert, 2020). Davey et al. (2003) also found that the vegan dietary pattern was high in vitamin B1, folate, vitamin C, vitamin E, iron, and magnesium when compared with non-vegan diet groups.

While there are numerous benefits to following a vegan diet, certain nutrients are not available, or are only available in small quantities, or are less bioavailable within the vegan diet. Vegans may have a low intake or status of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin B12, calcium, iodine, zinc and iron (Neufingerl & Eilander, 2021). Vitamin B12 is predominantly found in animal products (Australian Government Department of Health and Ageing & Ministry of Health,

2006) and research has shown that vegans who do not supplement B12 have significantly lower plasma cobalamin concentrations compared to non-vegans (Selinger, Kühn, Procházková, Anděl, & Gojda, 2019). Similarly, the omega-3s, especially EPA and DHA, are predominantly found in fish and seafood and intake is therefore lower in vegans (Pateiro et al., 2021). Conversion of alpha linolenic acid (ALA), which is found in plant food, to the more protective forms of DHA and EPA can take place but research has shown that high doses of ALA-containing foods may not increase the Omega-3 index in vegans (Lane, Wilson, Hellon, & Davies, 2022).

Another nutrient of concern is iron. Vegans may have a high iron intake due to the high iron content in some plant food and iron-fortified vegan food (Ancuceanu et al., 2015). However, haem iron, which is only found in animal products, has higher bioavailability when compared to non-haem iron found in plant foods (Bezwoda & Mayet, 1983; Layrisse & Martínez-Torres, 1972; Turnbull, Cleton, & Finch, 1962). For this reason, it is recommended that vegetarian and vegan's iron intake is 1.8 times higher than that of the recommended dietary intake (RDI) of omnivores (Australian Government Department of Health and Ageing & Ministry of Health, 2006).

Iron deficiency is the most prevalent micronutrient deficiency worldwide, especially in females of reproductive age (Percy, Mansour, & Fraser, 2017). Significant blood losses (e.g. blood donation, menstruation) or certain physiological conditions coupled with a diet low in iron, such as a vegan diet, are major risk factors for iron deficiency (Means, 2020). Iron deficiency anaemia (IDA) can result in poor pregnancy outcomes (impacting both the mother and offspring), low work productivity and seriously impact quality of life (Abbaspour, Hurrell, & Kelishadi, 2014).

Iron deficiency has major implications for individuals, healthcare systems and productivity globally (Miller, 2013). Worldwide, IDA results in 841,000 deaths and 35,057,000 disability adjusted life years (DALY) lost annually (Stoltzfus, 2003). Iron deficiency was identified as one of the five leading causes of years lived with disability in the latest Global Burden of Disease study (Vos et al., 2017). It is also the number one cause of DALY in women (Benson et al., 2021). Between 2017-2018 treating IDA in NZ cost $2.8million in prescribed supplements and $6.7million for hospitalisations (Ministry of Health, 2018).

Understanding the risk of ID for NZ vegans may help to prevent severe ID cases in this group. There is some evidence on the iron intake of vegans, which identified that iron intake was high amongst vegans (Allès et al., 2017; Clarys et al., 2014; Davey et al., 2003; Elorinne et al., 2016; García-Morant et al., 2020; Kristensen et al., 2015; Li, Sinclair, Mann, Turner, & Ball, 2000; Nebl et al., 2019; Schüpbach, Wegmüller, Berguerand, Bui, & Herter-Aeberli, 2017; Waldmann, Koschizke, Leitzmann, & Hahn, 2004; Weikert et al., 2020; Wilson & Ball, 1999). Less is known about how the diet impacts iron

status, how their iron status compares to that of other dietary population groups, and the long-term health effects. There is also no research on iron intake and status in a NZ vegan sample population.

The purpose of this study is to investigate iron intake and status of a sample of NZ vegans and explore potential risk factors for ID. The findings of this study could help inform nutrition guidelines and supplementation recommendations for NZ vegans.

1.1 Aims

To investigate iron intake and determine iron status and risk factors for ID in a NZ vegan sample population.

1.1.1 Objectives

1. Assess dietary intake of iron and iron enhancers/inhibitors intake in a NZ vegan sample population.
2. Analyse iron status in a NZ vegan sample population.
3. Compare dietary intake of iron and enhancers/ inhibitors of iron (vitamin C, calcium, dietary fibre and caffeine) in ID and iron sufficient individuals in a NZ vegan sample population.
4. Investigate differences in other potential risk factors of iron status Between ID and iron sufficient individuals in a NZ vegan sample population.

1.1.2 Hypotheses

1. It is hypothesised that the mean intake of iron in the NZ vegan sample will be high or sufficient (>8mg/day for male, >8mg/day for females over 50 years and, >18mg/day for females 19-50 years).
2. Overall, rates of ID (Serum ferritin (SF) <30μg/L) will be high and menstruating females will have the highest rates of ID.
3. No difference in the intake of iron and iron enhancers/inhibitors will be found in ID and iron sufficient participants.
4. Non-dietary determinants will be more strongly associated with ID compared to intake of iron and iron enhancers/inhibitors.

1.2 Structure of Thesis

The introduction, Chapter 1, highlights the prevalence and growth of veganism worldwide and in NZ and explores the implications of this dietary pattern outlining why further research on the determinants of iron status in NZ vegans is needed. Chapter two is a literature review that explores ID and the general and specific risks to NZ vegans, including current research on vegan iron intake and status. Chapter three is a manuscript for publication which includes the study methodology, results, and discussion of findings. The final section, Chapter 4, summarises the research, the strengths and limitations of the study, and how the findings contribute to the field and future directions for research.

1.3 Researcher's Contributions

Table 1.1 Summary of Researcher's Contributions to Study

Author	Contribution to Thesis
Amelia Dunnett Master of Science (MSc) Nutrition and Dietetics Student	Primary author Assistance with participant recruitment, data collection and entry of participant food diaries into FoodWorks, statistical analysis, writing, editing and final preparation of the thesis.
Dr Kathryn Beck Associate Professor of Human Nutrition	Primary Supervisor Revised and approved thesis. Assistance with statistical interpretation. Advisor regarding FoodWorks data entry.
Dr Claire Badenhorst Senior Lecturer in Exercise and Sport	Co-Supervisor Revised and approved thesis.
Dr Pamela von Hurst Professor of Human Nutrition	Primary investigator on the vegan study. Designed research study, applied for funding and ethics. Oversaw data collection.
Dr Hajar Mazahery Study Manager	Participant recruitment, data collection and review and development of the FoodWorks database.
Rebecca Paul Research Assistant	Participant recruitment, data collection and review and development of the FoodWorks database.
Owen Mugridge Project Co-ordinator	Participant recruitment and data collection.
Fellow MSc students Abril Clark, Rebecca Pearce, Catherine Alice, Chelsea Corkindale, Fiona Li, Lucie L'Estrange Hill	Participant recruitment, data collection and entry of the 4D-FR into FoodWorks.

Chapter 2: Literature Review

2.0 Introduction

It is estimated that 1.1% of the New Zealand population are following a vegan diet (Milfont et al., 2021). There are numerous studies that have investigated the health benefits of a vegan dietary pattern; however, studies investigating dietary intake and nutritional status in this population are less abundant (Neufingerl & Eilander, 2021). There are many nutrients that are less bioavailable or simply impossible to obtain from food alone on a vegan diet, one such nutrient is iron (Lynch et al., 2018). On a vegan diet, iron is only available in the form of non-haem iron, which is absorbed at a lower rate than haem iron, and consequently may increase the risk of developing iron deficiency (ID) (Pawlak, Berger, & Hines, 2018).

In individuals symptoms of ID include fatigue, reduced strength and exercise capacity, loss of hair, decreased ability to concentrate and impaired work performance (Soppi, 2018). If ID worsens there is increased risk of tachycardia and cardiac failure (Percy et al., 2017). In pregnancy, untreated ID could impair development of the foetal brain and other tissues and lead to cognitive impairment in the child and it is also one of the leading causes of anaemia in infants and young children (Abu-Ouf & Jan, 2015; Black, Quigg, Hurley, & Pepper, 2011).

2.1 Metabolism of Iron in Humans

Iron is an essential nutrient used in many processes in the human body including oxygen transport, deoxyribose nucleic acid (DNA) synthesis and cellular replication and repair, energy metabolism and innate immunity (Piskin, Cianciosi, Gulec, Tomas, & Capanoglu, 2022). It is crucial for red blood cell (RBC) production, also known as erythropoiesis. Around two thirds of iron in the human body is found in haemoglobin (Hb), the oxygen-transport metalloprotein found in erythrocytes (Alsadig, Almahdi, & Khalid, 2021). The synthesis of haem, the oxygen-binding component of Hb, relies on the intracellular availability of iron (Chung, Chen, & Paw, 2012). The demand for iron and haem increases as the erythrocyte matures (Camaschella, Pagani, Nai, & Silvestri, 2016). Iron is also utilised in proteins involved in DNA replication and repair (Sangkhae & Nemeth, 2017). Iron-sulphur (Fe-S) domains in these enzymes are thought to provide structural stability and facilitate their interactions with other proteins or nucleic acids (Zhang, 2014). Fe-S clusters are also essential components in oxidative phosphorylation (Read, Bentley, Archer, & Dunham-Snary, 2021). Fe-S co-factors are found within complexes I,II, and III in the electron transport chain (ETC), a cellular process that leads to the production of Adenosine Triphosphate (ATP) (Read et al., 2021). Iron is a key substrate in immune

function, although the mechanisms are less well understood (Cherayil, 2010). Iron can also have a direct effect on infection, acting as a nutrient for microbial pathogens, and it is therefore important that homeostasis is tightly regulated (Sangkhae & Nemeth, 2017). Free iron is also highly reactive and toxic because of its ability to readily accept or donate electrons (Ganz, 2013). Iron is therefore involved in the formation of reactive oxygen species (ROS) and the excessive accumulation of iron and ROS is linked to cell damage, death and is associated with acute and chronic degenerative conditions (Dixon & Stockwell, 2014).

Iron absorption is controlled at the enterocyte which is fundamental to iron homeostasis due to mechanisms of excretion being insufficient (Sangkhae & Nemeth, 2017). Hepcidin, a peptide hormone produced in the hepatocytes, is a key regulator of iron absorption in the enterocyte, it also controls the release of iron from cells that store and recycle iron, such as macrophages (Collins, Wessling-Resnick, & Knutson, 2008). Extracellular iron sensors feedback to the liver (hepatocytes) to induce hepcidin transcription in proportion to the concentrations of iron in the body. While less is known about intracellular feedback it has been proposed to occur via the bone morphogenic-SMAD (BMP-SMAD) pathway (Sangkhae & Nemeth, 2017).

Despite the tight control of enterocyte iron absorption, most of the body's iron needs are met through erythrophagocytosis (~19mg/d), where iron is recycled via phagocytosis of senescent RBC (Sukhbaatar & Weichhart, 2018). The remaining 1-2mg/d is absorbed from the diet (Piskin et al., 2022). Dietary iron is absorbed via the enterocytes of the small intestine, specifically the duodenum and upper jejunum (Gulec, Anderson, & Collins, 2014). There are two forms of iron found in food, haem and non-haem, and both are absorbed via different routes but enter the same intracellular pool of iron (Piskin et al., 2022). In food, non-haem iron is in the ferric (Fe^{3+}) form and to be absorbed it needs to be reduced to its ferrous (Fe^{2+}) form (Gulec et al., 2014). Reduction occurs through interactions with other nutrients or via interaction with the ferric reductase (duodenal cytochrome B) found on the enterocyte (Beard & Han, 2009). Once reduced, Fe^{2+} is transported into the cell via the Fe^{2+} iron transporter, divalent metal transporter (DMT1) (Andrews, 1999).

Haem iron is absorbed much more efficiently than non-haem iron, but less is known about its mechanism of absorption. Haem iron needs to be proteolyzed from Hb and myoglobin in the stomach and small intestine to be absorbed into the enterocyte (Anderson, Frazer, McKie, Vulpe, & Smith, 2005). The alkaline lumen of the small intestine means haem is soluble and does not require binding proteins for absorption (Gulec et al., 2014). It is hypothesized that haem is also transported into the enterocyte via specific transporter proteins (Flanagan, 1989). One example is the haem carrier protein (HCT1), believed to transport haem iron because it is highly expressed on the duodenal enterocyte

(Beard & Han, 2009). Once absorbed, haem is degraded by haem oxygenase-1 (HO-1) and Fe^{2+} iron is released and follows the same cytosolic pool of iron as non-haem iron (Raffin, Woo, Roost, Price, & Schmid, 1974). From here, iron is either stored in the enterocyte as ferritin or transferred across the basolateral membrane via ferroportin, into circulation, and is immediately oxidised from Fe^{2+} to Fe^{3+} so it can bind transferrin (Tf) (Flanagan, 1989). Transferrin, a transport protein, can bind two Fe^{3+} atoms and delivers iron to cells expressing Tf receptors (TfR1)(Knutson, 2017).

2.2 Iron Deficiency

There are several stages of ID, from ID no anaemia (IDNA) to IDA, that can affect iron utilization, the production of RBC, and severely affect quality of life. Absolute ID is where body iron stores are low, iron stores in macrophages and hepatocytes are reduced and, if left untreated, may advance to IDA (Cappellini, Musallam, & Taher, 2020). Conversely, functional ID, a common diagnosis in patients with acute and chronic infection or disease, occurs due to inflammation-mediated increases in hepcidin which prevent cellular iron export to the plasma (Thomas et al., 2013). In this condition, iron stores and SF will present as elevated but Tf saturation (TSAT) may decline, leading to ID erythropoiesis and IDA (Pasricha, Tye-Din, Muckenthaler, & Swinkels, 2021). Chronic functional ID may exacerbate the risk of absolute ID through hepcidin-mediated reduction of iron absorption (Cappellini et al., 2020).

Nutritional ID occurs when there are no other underlying causes for the depletion of iron stores and can be categorised into three stages (Lynch et al., 2018). Initially storage iron is depleted; serum ferritin (SF) is low but TSAT, red blood cell protoporphyrin, soluble Tf receptor (STfR), and Hb concentrations are all normal (Bermejo & García-López, 2009). Later, iron-deficiency erythropoiesis occurs and iron availability for erythropoiesis starts to become insufficient; serum iron and TSAT become low in addition to low SF, RBC protoporphyrin and STfR are increased, and Hb remains normal (Pfeiffer & Looker, 2017). The final stage is ID where SF, TSAT and Hb are low and RBC protoporphyrin and STfR are increased (Bermejo & García-López, 2009).

2.2.1 Diagnosing Iron Deficiency

Table 2.1 provides an overview of the symptoms and biomarker changes in different stages and conditions of ID. The current biomarkers are fairly accurate in diagnosing ID with haematological consequences, however, may not be as effective at capturing other known functional outcomes (Lynch et al., 2018).

The World Health Organization (WHO) defines IDA as a Hb concentration less that 130g/L in men and 120g/L in non-pregnant women over 15 years old (World Health Organization, 2015). However, data supporting these Hb thresholds to define anaemia are limited (Cappellini et al., 2020).

Serum ferritin is the most used biomarker to assess iron status in healthy individuals. It is a reliable measure to diagnose ID and has the best correlation with bone marrow iron staining (the "gold standard"); as well as being standardised and the most widely available test (Grote Beverborg et al., 2018). Ferritin concentration less than <15µg/L are specific for ID, and probable IDA, and concentrations <30µg/L coupled with symptoms mean ID is likely present (Pasricha et al., 2021). The higher cut-off of <30µg/L is associated with a higher sensitivity but may present with some false positive cases, however, the lower threshold of <15µg/L may miss up to 50% of positive cases (Daru et al., 2017; Soppi, 2018). Serum ferritin is not a reliable marker of ID when inflammation is present. During inflammation apoferritin, a form of ferritin, and hepcidin is increased leading to sequestration of iron in the enterocyte, macrophages, and hepatocytes and SF concentrations may present as normal or high (Cappellini et al., 2020). Assessing for the presence of inflammation using c-reactive protein (CRP), especially when inflammation or functional ID is suspected, is useful when using SF as a diagnostic marker (Dignass, Farrag, & Stein, 2018). A SF cut-off of 70µg/L in adults may be used to diagnose ID when inflammation (i.e., raised CRP (>5mg/L) is present (World Health Organization, 2020).

Biomarkers not directly influenced by inflammation include TSAT and STfR. Transferrin saturation indicates the iron available for erythropoiesis and delivery to the cells and is a useful indicator of iron status in the presence of inflammation (Dignass et al., 2018). TSAT concentrations <20% suggest low plasma availability of iron for tissues in both absolute and functional ID. Serum Tf is a biomarker for erythrocyte precursors (Cappellini et al., 2020). Although not directly influenced by inflammation it becomes less reliable as inflammation increases; interpretation is also complicated by the effect of age, ethnicity and altitude on STfR (Camaschella, 2017).

Table 2.1 *Biomarkers and Symptoms of Iron Deficiency at Different Deficiency States*

	Normal Iron Stores	Low Iron Stores	Absolute ID (IDNA)	Absolute ID (IDA)	Functional ID	Functional ID with Absolute ID
Symptoms	n/a	Mild fatigue or no symptoms	Fatigue, poor concentration, dizziness, tinnitus, headache, pica, and/or restless leg	Fatigue, poor concentration, dizziness, tinnitus, headache, pica, and/or restless leg.CV implications if severely low	Fatigue, poor concentration, dizziness, tinnitus, headache, pica, and/or restless leg	Fatigue, poor concentration, dizziness, tinnitus, headache, pica, and/or restless leg
Haemoglobin (Hb)	M: >130g/L F: >120gL	M: >130g/L F: >120gL	M: > or <130g/L F: > or < 120gL	M: <130g/L F: <120gL	M: <130g/L F: <120gL	M: <130g/L F: <120gL
Ferritin	>30 µg/L	15-30 µg/L	15-30 µg/L	<15-30 µg/L	Depending on inflammation may be normal or high	<70-100 µg/L depending on inflammation
TSAT	>20%	Usually >20%	<20%	<15%	Usually >20%	<20%
STfR	Normal	Normal	Increased	Increased	Normal	Normal or increased

TSAT – transferrin saturation; STfR – soluble transferrin receptor; CV – cardiovascular
M – male; F - female
Table adapted from (Pfeiffer & Looker, 2017)

2.2.2 Causes of Iron Deficiency and At-Risk Groups

Iron deficiency occurs when intake or absorption of iron is insufficient to meet physiological needs or to cover excessive blood losses (Cappellini et al., 2020). Poor iron intake and ID is common in developing countries due to food insecurity and malnutrition (Eicher-Miller, Mason, Weaver, McCabe, & Boushey, 2009). Inadequate absorption may also arise from a diet low in bioavailable iron, such as a vegetarian or vegan dietary pattern (Pawlak et al., 2018).

Absorption is also impaired in states of inflammation or infection (Collins et al., 2008). As such, studies have found a significant correlation between ID (based on serum iron and TSAT, not SF) and obesity (Pinhas-Hamiel et al., 2003; Zhao et al., 2015). As discussed in section 2.1.1, hepcidin is increased in states of inflammation, such as obesity, thus decreasing iron available for pathogens but also limiting the iron availability for erythropoiesis, which if not detected may eventually lead to functional ID (Qiu et al., 2022). As a result of this inflammation mediated hepcidin pathway, individuals with other inflammatory conditions or digestive disorders/disease are also at higher risk for ID (Bermejo & García-López, 2009).

Blood donation has also been identified as a predictor of ID (Beck et al., 2014; Cançado, Chiattone, Alonso, Langhi Júnior, & Alves, 2001; Fillet et al., 2021; Røsvik, Ulvik, Wentzel-Larsen, & Hervig, 2009). The body's iron stores are 300-800mg for men and women, with women and especially menstruating women being on the lower end of this range (Cook, Flowers, & Skikne, 2003). Each whole blood donation results in 200-300mg of iron lost (Fillet et al., 2021). This loss and the limited capacity for absorption and iron storage leads to a high risk for developing ID following blood donation, especially in women (Alvarez, Kirchner, Klüter, & Schlenke, 2000).

In New Zealand, pre-menopausal women have the highest prevalence of ID (University of Otago & Ministry of Health, 2011). Women of reproductive age are at high risk of ID due to regular menstrual blood losses, especially those with heavy menstrual bleeding (HMB). Therefore the recommended dietary intake (RDI) of iron for menstruating women is double that of men and non-menstruating women (Mansour, Hofmann, & Gemzell-Danielsson, 2021). Heavy menstrual bleeding (HMB) or menorrhagia is excessive menstrual bleeding of 80mL or more in a cycle that could continue for 7 days or more (Hallberg, Hôgdahl, Nilsson, & Rybo, 1966). HMB is estimated to affect 18-38% of women of reproductive age and may increase as females progress through perimenopause (Magnay, O'Brien, Gerlinger, & Seitz, 2018). A European survey identified that 63% of those who have HMB had experienced ID or IDA (Fraser et al., 2015).

In youth, ID is most likely to occur in infancy and adolescence. Iron requirements are high due to the speed of growth and development at these stages (Moscheo et al., 2022). A New Zealand study found that 14% of urban Auckland children 6-23 months old are iron deficient (Grant, Wall, Brunt, Crengle, & Scragg, 2007). At birth, iron stores are usually sufficient up until around 4 months and if iron intake and/or absorption is inadequate ID is likely to develop due to rapid growth and increased iron needs (Brunt, Grant, Wall, & Reed, 2012). In adolescents, the increasing blood volume and muscle mass associated with rapid growth is also associated with increase their iron needs (Mesías, Seiquer, & Navarro, 2013), which is reflected in the higher RDI for this cohort.

During pregnancy iron requirements of the female also increase. This is due to the need of iron in foetal development and to compensate for the loss of blood at delivery (Bothwell, 2000). If the mother is ID during pregnancy there is an increased risk that the infant's will develop ID too (Abu-Ouf & Jan, 2015).

Table 2.2 displays the RDI of iron for adults. Healthy adult men and menopausal women are less likely to become ID because intake from the diet is usually sufficient to cover their physiological needs (Abbaspour et al., 2014).

Table 2.2 Recommended Dietary Intake (RDI) & Estimated Average Requirements (EAR) of Iron for Adults

	Men	Women	Pregnancy	Lactation
19-50 years				
RDI	8mg/day	18mg/day	27mg/day	9mg/day
EAR	6mg/day	8mg/day	22mg/day	6.5mg/day
51-70+ years				
RDI	8mg/day	8mg/day	n/a	n/a
EAR	6mg/day	5mg/day	n/a	n/a
Table adapted from (Australian Government Department of Health and Ageing & Ministry of Health, 2006)				

These recommendations are based off a mixed western diet that contains animal foods and absorption of iron from this diet is assumed to be 18% (Australian Government Department of Health and Ageing & Ministry of Health, 2006). Australian Government Department of Health and Ageing & Ministry of Health (2006) state, however, that vegetarians' absorption rate is 10% or lower and it is therefore recommended that their RDI for iron should be 80% higher than the recommendations in table 2.2.

2.3 Bioavailability of Iron & Nutrient Interactions

2.3.1 Haem and Non-Haem Iron

There are two main forms of iron, haem and non-haem. Haem iron is only found in meat and animal products, such as milk and eggs, and is more bioavailable in comparison to non-haem (Trumbo, Yates, Schlicker, & Poos, 2001). In the alkaline environment of the intestine, haem is soluble and does not require any binding proteins to be absorbed, it is also less affected by polymerisation (Ma, Kim, & Han, 2010). Unlike non-haem iron, haem absorption is unaffected by humic substances (e.g. tannins and phytates) and chelators (Anderson et al., 2005). Foods containing haem iron are high in protein, which also increases the solubility of haem (Raffin et al., 1974). Non-haem iron is found in both plant foods and meat (Neufingerl & Eilander, 2021). The absorption rate on non-haem iron is variable and depends on the iron status of the individual and the presence of inhibitors and enhancers in the food (Hurrell & Egli, 2010; Lynch et al., 2018; Neufingerl & Eilander, 2021). The reduced form of iron, Fe^{2+} (ferrous), is absorbed via the enterocyte, however, the predominant form of iron found in food is the oxidized Fe^{3+} (ferric) (Piskin et al., 2022). Ferric iron must be reduced to be absorbed in the alkaline environment of the small intestine lumen; which occurs through interactions with chelating agents in food, such as ascorbic acid, or contact with duodenal cytochrome B on the apical surface (Ma et al.,

2010; Neufingerl & Eilander, 2021). Chelators can either enhance or inhibit iron absorption by affecting its solubility (Hatcher, Singh, Torti, & Torti, 2009).

2.3.2 Iron Absorption Enhancers

Single meal studies have demonstrated that ascorbic acid has a significant effect on the absorption of iron (Ballot et al., 1987; Cook & Monsen, 1977; Hallberg, Brune, & Rossander, 1986; Thankachan, Walczyk, Muthayya, Kurpad, & Hurrell, 2008). Ascorbic acid forms a chelate complex with Fe^{3+} and accelerates the redox reaction to Fe^{2+} (Timoshnikov, Kobzeva, Polyakov, & Kontoghiorghes, 2020). The chelation also reduces the inhibitory effects of ligands in the diet such as phytates and tannins (Hallberg et al., 1986).

However, complete meal studies are contradictory and the effect on absorption may not result in improved iron status over the longer term. One complete meal study found that the effect of ascorbic acid on iron absorption was much smaller than that demonstrated in single meal studies (Cook & Reddy, 2001). While a systematic review found ascorbic acid supplementation increased iron absorption significantly (Collings et al., 2013), in longer-term studies, a significant effect of ascorbic acid supplementation on SF concentrations or iron status overall has not been demonstrated (Cook, Watson, Simpson, Lipschitz, & Skikne, 1984; Garcia, Diaz, Rosado, & Allen, 2003; Li et al., 2020; Malone, Kevany, Scott, O'Broin, & O'Connor, 1986; Péneau et al., 2008). Similar to ascorbic acid, the addition of meat, fish or poultry to a vegetarian meal has been shown to enhance non-haem iron absorption in iron absorption studies (Baech et al., 2003; Navas-Carretero et al., 2008). It is thought that absorption is enhanced from the effect of the cystine-containing peptides that reduce and chelate iron, forming soluble complexes (Hurrell & Egli, 2010; Hurrell, Reddy, Juillerat, & Cook, 2006; Navas-Carretero et al., 2008).

Baech et al. (2003) found that the addition of 50g or more of pork-meat increased non-haem iron absorption in a phytate-rich and low vitamin C containing meal in healthy women subjects. Navas-Carretero et al. (2008) found that the addition of fish to a bean meal significantly increased iron absorption. Another study looked at the effect of a high meat-based diet compared to a vegetable-based diet intervention in 57 menstruating women with low iron stores (SF <30µ/L, haemoglobin <120 g/L) and found SF remained unchanged in the meat-based diet group whereas in the vegetable-based diet group SF declined from a baseline of 17.3 to 11.2µg/L ($P \leq 0.001$) after 20 weeks (Tetens, Bendtsen, Henriksen, Ersbøll, & Milman, 2007).

Other organic acids, such as citric acid, may also enhance iron absorption (Salovaara, Sandberg, & Andlid, 2002). However, ascorbic acid is likely the most effective due to its ability to reduce $Fe3^{+}$ to

$Fe2^{+}$, whereas other organic acids may be needed in higher concentrations to influence iron absorption but research is limited (Teucher, Olivares, & Cori, 2004).

Finally, alcohol consumption may increase the absorption of iron, with one study demonstrating decreased risk of ID and IDA in participants that consumed two alcoholic beverages per day (Ioannou, Dominitz, Weiss, Heagerty, & Kowdley, 2004).

2.3.3 Iron Absorption Inhibitors

Other proteins may be inhibitory to iron absorption. One example would be soy proteins that have been shown to inhibit iron absorption (Cook, Morck, & Lynch, 1981; Lynch, Dassenko, Cook, Juillerat, & Hurrell, 1994). Lynch et al. (1994) found that both phytate and conglycinin, protein-related moieties, found in soybean-protein are inhibitory to iron absorption. Significantly reducing the phytate content did not enhance iron absorption from the conglycinin meal; whereas, reduction of phytates in the meal containing the glycinin fraction increased absorption nearly 6-fold (Lynch et al., 1994). Egg albumin has also been shown to inhibit iron absorption, although the mechanism is poorly understood (Hurrell, Lynch, Trinidad, Dassenko, & Cook, 1988; Monsen & Cook, 1979)

Some forms of fibre, such as bran, may have an inhibitory effect on the absorption of iron, however, the inhibitory effect of fibre probably has less impact compared to other nutrients (Cook, Noble, Morck, Lynch, & Petersburg, 1983). An epidemiological study found that intake of fibre-poor fruits and vegetables was associated with higher SF in premenopausal women (Péneau et al., 2008). However, it is unknow if this is due to the lower fibre intake or reduced compounds within the fibre such as phytic acid, polyphenols, and oxalates.

Phytic acid is found in cereals, seed oils and legumes and can have a strong inhibitory effect on iron absorption (Hallberg, Brune, & Rossander, 1989; Marie Minihane & Rimbach, 2002). At the pH of the small intestine, phytic acid binds iron and forms an unabsorbable complex (Brouns, 2021). Ascorbic acid may overcome the inhibitory effects of phytate (Hallberg et al., 1989). A small single-meal study on men found that iron absorption was significantly higher when consuming a tortilla genetically modified to be low in phytic acid compared to a wild-type tortilla, around 35% higher in phytic acid (8.2% vs 5.5%, P=0.001) (Mendoza et al., 1998).

Polyphenols are found in tea, coffee, red wine, cocoa, spices, cereals, and vegetables. They form stable complexes with non-haem iron, affecting absorption into the enterocyte (Hurrell, Reddy, & Cook, 1999). A female-only study found removing phenolic acid and polyphenols from a bean meal increased iron absorption 2.6 fold ($p \leq 0.001$) and removal of polyphenols from dephytinized porridge doubled iron absorption rates ($p \leq 0.001$) (Petry, Egli, Zeder, Walczyk, & Hurrell, 2010).

Oxalates are found naturally in plant foods such as fruits, vegetables, nuts, seeds and grains (Milman, 2020). Oxalates have been shown to inhibit calcium absorption by forming insoluble calcium oxalates, however, it is unknown if oxalates have a significant effect on iron absorption in humans (Piskin et al., 2022). An in-vitro study did find strong inhibitory affects (Gupta, Lakshmi, & Prakash, 2006). Although, these effects were not found in a human study that compared a kale containing meal (low in oxalates) to a spinach containing meal (high in oxalates), where no significant difference in iron absorption was reported (Storcksdieck, Walczyk, Renggli, & Hurrell, 2008).

Finally, calcium may inhibit iron absorption and there are two proposed mechanisms. It may promote internalisation of the DMT1 receptors or, because it has an effect on both non-haem and haem iron absorption, thus interrupts iron transport from the enterocyte into circulation via the basolateral membrane (Abioye et al., 2021). The inhibitory effect of calcium alone on iron absorption has been demonstrated with doses of 300-600mg, decreasing absorption by up to 60% in both non-haem as well as haem iron containing meals (Hallberg, Rossander-Hultén, Brune, & Gleerup, 1992). A recent systematic review and meta-analysis found an inverse relationship between calcium intake and iron status in the short-term (<90 days), however, they also found no effect in longer-term studies (Abioye et al., 2021).

Most iron-absorption studies are in-vitro or single-meal studies, and the effect of enhancers and inhibitors may be amplified. The research is mostly short-term, and a lot of intra-individual variation has been observed making it difficult to infer the effects of iron enhancers and inhibitors to guide population recommendations. Methodological challenges make it difficult to measure iron absorption in humans, which limits the quality research available leaving an incomplete understanding of iron absorption and bioavailability (Ancuceanu et al., 2015).

2.4 Measuring Dietary Intake in Populations

The aim of nutrition epidemiological research is to investigate associations between dietary intake and health-related outcomes (Naska, Lagiou, & Lagiou, 2017). It is challenging to gain accurate dietary assessments; accuracy needs to be balanced with the appropriate method that is viable for large populations and specific groups of people (Bingham et al., 1994).

Three methods are commonly used in epidemiological research: food frequency questionnaires (FFQ), food record (FR), and 24-hour dietary recall (24HR). The 24HR is a thorough interview carried out by a trained researcher and gathers 24 hours of an individual's food and beverage intake, which is usually from the day prior, and typically takes about 20-30 minutes to complete (Shim, Oh, & Kim, 2014). The advantages of this method include the detailed record that is obtained, which includes brands and

portion sizes, with minimal participant burden (Carroll et al., 2012). However, the individual's memory and interviewer's skill affects the quality of information and recall bias (Bingham et al., 1994).

The FR requires the individual to record all food and beverage consumed, concurrently, for a specified period (minimum of 3 days). Portions are either estimated or weighed and the individual is trained prior to commencing recording. The FR is stronger at estimating energy and protein intake compared to other methods (Bingham et al., 1994) (Prentice et al., 2011). A 12 day weighed FR has been shown to estimate an individual's iron intake to within 10% of mean typical intake (Heath, Roe, Oyston, & Fairweather-Tait, 2005). However, the participant burden is higher, especially with an increased recording period, and needs to be considered in the study design as it can affect accurate recording and compliance (Ortega, Pérez-Rodrigo, & López-Sobaler, 2015).

The 24HR and FR allow detailed information to be collected, and they can be applied to a wide range of groups (Carroll et al., 2012). However, single measurement only provides short-term intake data, which doesn't account for seasonal variability; as a result repeated measurement is required to measure average exposures accurately, which is time and resource expensive (Shim et al., 2014).

Conversely, the FFQ gathers long-term dietary intake data. Participants are asked how much and how frequently they consumed certain foods or beverages over a specific period, usually a year (Shim et al., 2014). The FFQ is frequently used in epidemiological research because they are less burdensome for participants, taking about 20-30 minutes, and it does not require trained researchers to administer (Steinemann et al., 2017). However, the FFQ must be developed for the specific study population because factors such as ethnicity, culture and economic status will influence diet (Shim et al., 2014). It also needs to be validated and tested for reliability to ensure accuracy (Noor Hafizah et al., 2019). The FFQ is designed to report average intake over a longer period, however, it may emphasize recently consumed foods and, therefore, alter quantities of food groups consumed depending on the season (Fowke et al., 2004).

All recall method, such as the FFQ, are prone to measurement error as individuals need to recall intake over a longer period of time (Bailey, 2021). Misreporting is an issue with inaccurate reporting of some foods for social reasons i.e., wanting to please the interviewer and reporting lower intakes of less "socially desirable" and higher intakes of foods / nutrients that are more "acceptable", such as protein. As a result, protein intake has been found to be substantially over-estimated in all 3 dietary assessment methods (Kipnis et al., 2002).

Another issue with all three methods is that the individuals' records need to be entered into a food composition database, where actual weights need to be interpreted and foods need to be closely

matched with similar foods available in the database. This can be time consuming (particularly with the 24HR and FR) and result in variation from what the individuals report due to certain food items not being available in the database and interpretation of unclear information (Shim et al., 2014).

Finally, assessing supplement intake is important because they can provide a high concentration of micronutrients, but measurement is challenging. Measurement can be carried out using the recall techniques described above (Bailey, 2021). The issue is there is no standardised technique and less is known about the error in self-reporting (Bailey et al., 2019). Bailey et al. (2019) discuss three key issues with assessing supplement intake; supplements may be consumed daily or episodically, labels on supplements use ingredients that differ to the form of micronutrients found in food, and the bioavailability of micronutrients from supplements may differ to bioavailability from food.

All three methods are prone to measurement error; however, they are still very important in epidemiological research and methods are being refined all the time to improve record accuracy. This includes the use of technology such as web-based programmes, mobile applications and wearable devices that enable easier collection and reduce the cost and time of processing the collected information (Eldridge et al., 2018). Each method has its benefits and limitations and using a combination of methods can help mitigate the measurement error (Bailey, 2021).

2.5 Research on Iron Intake and Status in Vegans

2.5.1 Dietary Iron Intake in Vegans

Previous research suggests iron intake in vegans is high, as summarised in tables 2.3 and 2.4. Four of the reviewed studies included supplement intake in their dietary intake analysis, and iron intakes for three out of four were well above EAR (García-Morant et al., 2020; Kristensen et al., 2015; Nebl et al., 2019; Wilson & Ball, 1999). Even the studies that did not include supplements in their analyses found average iron intake, from food alone, in vegans was higher than the RDI (Australian Government Department of Health and Ageing & Ministry of Health, 2006) (Allès et al., 2017; Clarys et al., 2014; Elorinne et al., 2016; Li et al., 2000; Schüpbach et al., 2017; Waldmann et al., 2004)

Using a 24HR and including supplement intake, García-Morant et al. (2020) found the highest average iron intake (28±10 mg/day) in women on a vegan diet. However, they only used a single 24HR which possibly overestimated iron intake. Ideally, multiple 24 hour recalls should be collected on non-consecutive days to acount for sigificant variation in day-to-day nutrient intake (Bailey, 2021). In Denmark, Kristensen et al. (2015) found considerably lower average iron intakes in women, especially when supplements were not included, (without supplements: 13.5mg/day; with supplements: 16mg/day). Fortified foods were prohibited by law in Denmark until 2003 and were still not widely

available at the time of the study, which may explain the relatively lower iron intake (Buch-Weeke, 2014). Similarly, the Epic-Oxford vegan sample (United Kingdom) also found lower iron intakes in vegans (M: 15.3mg/day; F: 14.1mg/day). However, the FFQ used possibly underestimated energy intake, and consequently most nutrients, because uniform portion sizes were assigned for men and women. This may be especially pertinant to vegetarians and vegans who may eat larger portions of carbohydrate foods (Davey et al., 2003).

2.5.2 Dietary Iron Intake in Vegans Compared to Non-Vegans

In most studies iron intake was highest in vegans compared to omnivores and/or vegetarians. Four studies found iron intake in vegans was significantly higher than the omnivore groups (García-Morant et al., 2020; Kristensen et al., 2015; Schüpbach et al., 2017; Weikert et al., 2020). Another three studies also found that vegans had a higher intake compared to omnivores as well as lacto-ovo vegetarians (Allès et al., 2017; Clarys et al., 2014; Nebl et al., 2019). Two studies in Australia compared iron intake in vegan men to other male diet groups (Li et al., 2000; Wilson & Ball, 1999). Li et al. (2000) found that vegans had a higher iron intake than both lacto-ovo vegetarians and moderate meat-eaters but not high meat-eaters. Wilson & Ball (1999) found that vegan's iron intake was significantly higher than omnivores. Only Elorinne et al. (2016) found no significant difference between iron intake in vegans and the comparison diet groups. However, there was a considerable difference in intake (vegans (n=22): 21mg/day versus non-vegetarians (n=15): 15mg/day; bonferroni correction was used for multiple corrections ($p \leq 0.0016$) and the p-value of 0.026 was not considered significant.

2.5.3 Iron Status in Vegans Compared to Vegetarians and Omnivores

Tables 2.4 and 2.5 display the average SF concentrations of vegans compared to non-vegans. The studies that analysed iron status in vegan males and females combined found that average SF concentrations ranged from 26-60µg/L (Elorinne et al., 2016; Schüpbach et al., 2017; Weikert et al., 2020). In all four studies that analysed iron status in vegan females separate to males, average SF concentrations in females were <30µg/L (Gallego-Narbón, Zapatera, & Vaquero, 2019; Henjum, Groufh-Jacobsen, Stea, Tonheim, & Almendingen, 2021; Obeid, Geisel, Schorr, Hübner, & Herrmann, 2002; Waldmann et al., 2004). Three of the four studies that compared SF concentrations in males compared to females found males had significantly higher SF than females, however, when they compared males and female vegans (separately) to the other diet groups all studies found no significant difference in SF between the groups (Gallego-Narbón et al., 2019; Henjum et al., 2021; Obeid et al., 2002). The studies that compared males and females (combined) found no significant

difference between SF in vegans compared to non-vegans (Elorinne et al., 2016; Schüpbach et al., 2017; Weikert et al., 2020).

Interestingly, only two studies found a significant difference in SF concentration between the different diet groups and both had only male participants (Li et al., 2000; Wilson & Ball, 1999). Li et al. (2000) found that SF in vegan males was significantly lower compared to males who ate moderate and high amounts of meat. Similarly, Wilson & Ball (1999) found SF and Hb concentrations of vegans (SF: 65µg/L, Hb:158g/L) was significantly lower than omnivores (SF: 121µg/L, Hb:173g/L). However, in both studies the vegan participant numbers were low (n=18 and n=10, respectively). In most studies that investigated iron status, the total numbers of vegan participants were n=≤50, and only one study had >100 participants. During the previous analysis, the vegan groups were often compared to different diet groups with much higher sample sizes. These factors potentially affect statistical power and limit the ability to observe a true effect (Zhao et al., 2021).

Pre-menopausal women who regularly menstruate are one of the highest risk groups for ID (World Health Organisation, 2011). Waldmann et al. 2004 grouped their participants (vegan only) into <50 years old (young women, YW), still menstruating, and ≥50 years old (old women, OW) and found SF was significantly lower in YW (YW: 14µg/L OW: 28µg/L) and 52% of YW were ID or IDA compared to 20% of OW. The SF of YW in this sample was much lower compared to the other 3 studies that looked at SF in females (where females of all ages were grouped together), however, average SF for females was still <30µg/L in all three (Gallego-Narbón et al., 2019; Henjum et al., 2021; Obeid et al., 2002).

2.5.4 Predictors of Iron Status in Vegans

Only two studies have investigated the correlations between iron intake and iron status and neither have found a relationship between iron intake and status in vegans (Schüpbach et al., 2017; Waldmann et al., 2004). Schüpbach et al., (2017) found that iron intake was positively correlated with plasma ferritin (PF) in omnivores and vegetarians but not vegans (r=0.168, p=0.281). Waldman et al. (2004) also found no correlation between SF and iron intake in vegans, nor iron absorption enhancers and inhibitors. Another two studies investigated other determinants of iron status in vegans (Gallego-Narbón et al., 2019; Henjum et al., 2021). Henjum et al. (2021) looked at sex, body mass index (BMI), age, education level, pregnancy, lactation, dietary practice, duration of dietary practice (vegan, vegetarian, pescatarian), use of supplements and smoking as predictors of iron status. They found only duration of dietary practice and being female were significant predictors of iron status. Gallego-Narbón et al. (2019) found no relationship between iron status and menstrual cycle and period length, heavy bleeding days, contraceptive use, and contraceptive type. Waist-to-hip ratio, overall muscle, abdominal muscle, and bone mass, however, were all observed to be higher in iron sufficient individuals.

Table 2.3 *Summary of Studies Investigating Iron Intake in Vegans Compared to Other Diet Group*

Author, Year, Country	Number of Vegan participants (n)	Age of Vegans (years)	Sample Population (n)	Duration Following Vegan Diet	Dietary Assessment Method	Included Supplements	Iron Intake in Vegans (mg/day)	Iron Intake in Vegans vs Other Diet Groups
García-Morant et al. 2020 Spain	102	18+	**M&F:** VG (102) OMN (DNS)	≥1 year	1x 24H	Y	F:28 (10) M:24 (9)	VG > OMN
Nebl et al. 2019 Germany	27	18-35	**M&F:** LOV (25) VG (27) OMN (27)	>6 months	3D FR	Y	Excluding supplements: 19.6(16.8, 22.4)mg/day Intake from supplements: 3.74(−0.64, 8.12)	VG > LOV VG > OMN
Kristensen et al. 2015 Denmark	70	18-61	**M&F:** VG (70) OMN (1,257)	≥1 year	4D weighed FR	Y	Excluding supplements: W:13.5(11.0-17.1) M:18.5(16.0-24.3) including supplements: F:16(13-20) M:23(18-30)	VG > OMN
Allès et al. 2017 France	789	18+	**M&F:** LOV (2,370) VG (789) OMN (90,664)	DNS	3x 24H	N	18.6(4.2)	VG > LOV VG > OMN
Clarys et al. 2014 Belgium	104	20-69	**M&F:** VG (104) LOV (573) S-V (498) PES (145) OMV (155)	DNS	FFQ	N	23(10)	VG > LOV VG > S-V VG > PES VG > OMN
Davey et al. 2003 United Kingdom	2,112	20-97	**M&F:** VG (2,112) LOV (18,840) PES (10,110) OMV (33,883)	DNS	FFQ	N	M:15.3(4.98) F:14.1(4.81)	VG > LOV VG > PES VG > OMN

M = male; F=female; VG = vegan; LOV = lacto-ovo vegetarian; S-V = semi-vegetarian; PES = pescatarian; OMN = omnivore; DNS = did not state; D = day; FR = food record; 24H = 24-hour food record; FFQ = food frequency questionnaire; SF = serum ferritin; PF = plasma ferritin; Hb = haemoglobin; ID = iron deficiency; IDA = iron deficiency anaemia
'Iron Intake in Vegans' displayed as mean (SD), mean (95% confidence interval) or median (interquartile range)

Table 2.4 *Summary of Studies Investigating Iron Intake and Iron Status in Vegans Compared to Other Diet Group*

Author, Year, Country	Number of Vegan participants (n)	Age of Vegans (years)	Sample Population	Duration Following Vegan Diet	Survey Method	Supplement use Included in Analysis	Iron Intake in Vegans (mg/day)	Iron Intake of Vegans Compared to Other Diet Groups	Iron Status in Vegans	Iron Status of Vegans SD to Other Diet Groups?	Prevalence of ID in Vegans	ID Definition
Weikert et al. 2020 Germany	36	30-57	**M&F:** VG (36) OMN (36)	DNS	3D weighed FR	N	22 (15.5–26.4)mg/day	VG > OMN	SF:60 (31–84)µg/mL	VG > OMN*	**ID:** n=4	SF F:<15µg/L M:<30µg/L
Elorinne et al. 2016 Finland	22	18-50	**M&F:** VG (22) OMN (19)	≥1 year	3D weighed FR	N	21(9)mg/day	VG > OMN*	SF:26(20, 39)µg/L Hb:139(122, 144)g/L	N	13.6% VG anaemic but DNS cause	DNS
Schüpbach et al. 2017 Switzerland	53	18-50	**M&F:** VG (53) LOV (53) OMN (100)	≥1 year	3D weighed FR	N	22.9(12.8–43.0)mg/day	VG > LOV VG > OMN	PF: 40(9–277)µg/L Hb: 147(11)g/L	N	0	SF <15µg/L
Waldmann et al. 2004 Germany	75	19-50+	**F:** Strict VG (43) Moderate VG (32)	≥1 year	2x 9D FFQ	N	YW:20(5.77)mg/day OW:19.6(5.14)mg/day	N/A	SF: YW:14(5, 84.6)µg/L OW:28(5, 70.5)µg/L	N/A	**ID:** YW:52% OW:20% **IDA:** YW:4% OW:4%	ID: SF <12µg/L IDA:TIM + Hb<120g/L
Li et al. 2000 Australia	18	21-50	**M:** VG (18) LOV (46) MME-OMN (65) HME-OMN (18)	≥6 months	semiquantitative FFQ	N	25.7(9.5)mg/day	VG > LOV VG > MME-OMN VG < HME-OMN	SF: 50(29)µg/L	VG = LOV VG < MME-OMN VG < HME-OMN	**ID:** n=2	SF <12 µg/L
Wilson et al. 1999 Australia	10	20-50	**M:** VG (10) LOV (39) OMN (25)	≥6 months	12D semiquantitative FR	Y	22.9(6.2)mg/day	VG = LOV VG > OMN	SF:65(50)µg/L Hb: 158(28)g/L	VG = LOV VG < OMN	**ID:** n=2	SF <12 µg/L

M = male; F=female; N = no; VG = vegan; LOV = lacto-ovo vegetarian; S-V = semi-vegetarian; PES = pescatarian; OMN = omnivore; MME-OMN = moderate meat eater; HME-OMN = high meat eater; DNS = did not state; N/A = not applicable; D = day; FR = food record; FFQ = food frequency questionnaire; SF = serum ferritin; PF = plasma ferritin; Hb = haemoglobin; ID = iron deficiency; IDA = iron deficiency anaemia
'Iron Intake in Vegans' and 'Iron Status in Vegans' displayed as mean (SD), mean (95% confidence interval) or median (interquartile range)

Table 2.5 *Summary of Studies Investigating Iron Status in Vegans Compared to Other Diet Group*

Author, Year, Country	Number of Vegan participants (n)	Age of Vegans (years)	Sample Population (n)	Duration Following Vegan Diet	Iron Status in Vegans	Iron Status of Vegans SD to Other Diet Groups?	Prevalence of ID	ID Definition
Henjum et al. 2021 Norway	106	18-60	M&F: VG (106) LOV (54) PES (31)	≥1 year	SF: F:27(19,43)µg/L M: 70(19,43)µg/L	N	DNS	SF F:<15µg/L M:<20µg/L
Gallego-Narbon et al. 2019 Spain	55	18+	M&F VG (55) LOV (49)	DNS	SF: F:21.9(16.1)µg/L M:71.4(19.1)µg/L	N	DNS	SF ≥15 to≤30 µg/L
Obeid et al. 2002 Germany & Netherlands	29	18+	M&F VG LOV (64) S-V (20)	DNS	SF: F:21(12–76)µg/L M:30(15–160)µg/L	N	37% total subjects and 43.8% of F ID	F:SF <15µg/L M:SF<30µg/L

M = male; F=female; N = no; VG = vegan; LOV = lacto-ovo vegetarian; S-V = semi-vegetarian; PES = pescatarian; DNS = did not state; SF = serum ferritin; PF = plasma ferritin; Hb = haemoglobin; ID = iron deficiency; IDA = iron deficiency anaemia
'Iron Status in Vegans' displayed as mean (SD), mean (95% confidence interval) or median (interquartile range)

2.6 Conclusion

The existing research on iron status in vegans indicates that dietary iron intake is high in this group, but it may not translate to high iron status. Prior research has demonstrated non-haem iron is less bioavailable than haem iron and therefore more iron is required for vegetarians (and vegans) to prevent ID. Most studies found that SF concentrations did not significantly differ between vegans and those following non-vegan diets. Two studies even found that SF was significantly lower in vegans despite significantly higher intakes of iron (Li et al., 2000) (Wilson & Ball, 1999). However, the research comparing iron intake to iron status is limited with only six studies available for review; all of which had sample sizes less than 100, used differing methods of dietary assessment and only two included supplement intake. To the best of our knowledge, there is no research on iron intake or status in a New Zealand vegan sample population. Further research with larger samples and standardised dietary assessment collection would improve the understanding of the relationship between the vegan diet and iron status. This understanding is important to identify risk factors and guide nutrition recommendations for vegans.

Chapter 3: Manuscript

3.0 Abstract

Background: It is estimated that 1.1% of New Zealanders are following a vegan diet. Despite there being many reported health benefits gained by following a vegan diet, vegans may be at higher risk for iron deficiency due to their dietary practice limiting the amount of bioavailable haem-iron. To the best of our knowledge, no studies have investigated iron intake and status in a NZ vegan sample.

Objectives: To investigate iron intake and iron status in a NZ vegan sample. This is inclusive of evaluating their risk for iron deficiency (ID) and potential risk factors of iron depletion in this vegan sample population.

Methods: The "Vegan Diet Research Programme", a cross-sectional Auckland-based study, recruited male (n=57) and female (n=155) vegan participants (n=212). Iron intake information, including nutrients that influence iron absorption, was gathered via a four-day food record (4D-FR). Iron status biomarkers were investigated, including serum ferritin (SF), haemoglobin (Hb) and C-reactive protein (CRP). Risk factors for ID were assessed via questionnaires. Participants were grouped into ID (SF <30µg/L) and iron sufficient (SF ≥30µg/L) and possible potential risk factors of iron status were compared.

Results: Overall, 47.3% of participants were ID, females (58.7%) had significantly higher rates of ID compared to males (14.2%) ($p \leq 0.001$). Those more likely to be ID were females ($p \leq 0.001$), younger individuals ($p \leq 0.001$), previously ID participants ($p \leq 0.001$), and those that had donated blood within the last six months ($p = 0.004$). Females had mean iron intakes (17.2 ± 5.2mg/day) below the NZ recommended dietary intake (RDI) (18mg/day) but higher than the estimated average intake (EAR) (5-8mg/day). In females, younger individuals ($p = \leq 0.001$), blood donation within the last six months ($p = 0.025$), and still menstruating ($p = 0.010$) were significant potential risk factors of ID. In males, only dietary intake was significantly different between ID and iron sufficient individuals. Energy (≤0.001), protein ($p = 0.004$), dietary fibre (≤0.001), iron ($p = 0.001$), calcium ($p = 0.003$) and vitamin C ($p = 0.006$) intake was significantly higher in ID males. However, only 8 males were ID and 6 of those returned food diaries.

Conclusion: This is the first study in NZ to analyse iron intake and status in a specifically vegan sample. Prevalence of ID was high overall, nearly half the sample was ID and over half of vegan females was ID. Iron deficiency was significantly high in females, younger individuals, individuals that had donated blood within the last six months and those previously diagnosed with ID. A relationship with dietary intake of iron and enhancers/inhibitors was found in males but not females. In females, blood donation within the last six months, menstruation and younger age were risk factors for ID. This study has provided novel insights to ID risk factors for a vegan population.

3.1 Introduction

Vegan diets are becoming increasingly popular. The number of individuals following a plant-based diet in the United States increased by nearly 10 million in the last 15 years and Google Trends and PubMed publications related to veganism have increased significantly (Milfont et al., 2021) (Medawar, Huhn, Villringer, & Veronica Witte, 2019). This trend may be partly due to increasing advice from government organisations and health authorities promoting a plant-based diet for improved health as well as promoting more sustainable food systems (Kumanyika et al., 2020; Willett et al., 2019). In NZ, the most recent estimated of prevalence of individuals following a vegan diet was 1.1%, representing 50-60,000 individuals (Milfont et al., 2021).

Research has shown that adherence to a vegan diet can have a number of health benefits, including improved metabolic status, decreased incidence of type 2 diabetes mellitus (T2DM) and mortality from ischemic heart disease, and significantly reduced overall incidence risk of cancers (Benatar & Stewart, 2018) (Kim et al., 2019) (McMacken & Shah, 2017) (Dinu et al., 2017) (Tantamango-Bartley et al., 2013). These health benefits may be explained by certain aspects of the vegan diet, including the lower total energy, lower saturated fat and higher dietary fibre intake (Bakaloudi et al., 2021; Benatar & Stewart, 2018; Davey et al., 2003; Neufingerl & Eilander, 2021). Intake of some micronutrients have also found to be higher in a vegan dietary pattern, including vitamin B1, folate, vitamin C, vitamin C, vitamin E, iron, and magnesium (Davey et al., 2003). However, some nutrients are less bioavailable, or not available at all (e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)). Of note, most research has provided short term insights on the health benefits of the diet and less is known about the long-term impact on health outcomes.

Iron is one such nutrient that may be less bioavailable on a vegan diet. Iron deficiency is the most prevalent micronutrient deficiency worldwide, especially in females of reproductive age (Percy et al., 2017). ID can result in poor pregnancy outcomes (for the mother and offspring), low work productivity and cumulative symptoms are strongly associated with reduced quality of life (Abbaspour et al., 2014). Treating ID in NZ costs $2.8 million in prescribed supplements and $6.7million for hospitalisations between 2017-2018 (Ministry of Health, 2018). An understanding of the iron intake and iron status of vegans and the implications of the vegan diet on iron status may help to prevent and identify at-risk groups for ID within NZ.

Previous studies in vegans have shown that iron intake tends to be high or at least meet the recommended dietary intake (RDI) thresholds in vegan samples (Allès et al., 2017; Clarys et al., 2014; Elorinne et al., 2016; García-Morant et al., 2020; Kristensen et al., 2015; Li et al., 2000; Nebl et al., 2019; Schüpbach et al., 2017; Waldmann et al., 2004; Wilson & Ball, 1999). However, the average reported intakes often do not meet the 1.8 times the RDI as recommended for vegans, especially for

menstruating women (Australian Government Department of Health and Ageing & Ministry of Health, 2006). Previous studies have compared the iron status of vegans to other diet groups (Li et al., 2000; Weikert et al., 2020; Wilson & Ball, 1999), with one study reporting that vegans had higher serum ferritin concentrations compared to omnivores (Weikert et al., 2020). Whereas, conflicting serum ferritin results were reported in another two studies which only recruited a small number of male vegan participants (Li et al., 2000; Wilson & Ball, 1999).

There is limited research on the iron intake and status in vegan sample populations and no NZ-specific research. This investigation aims to quantify the iron intake of a NZ vegan sample population, assess associations between nutrient intake and iron status, and compare iron status in relation to other known risk factors for ID. Findings will provide more evidence to guide nutrition and supplement recommendations regarding iron for vegans and enable effective screening for at-risk groups within the New Zealand population.

3.2 Methodology

The relationship between iron status and diet in New Zealand vegans was undertaken as a part of the Vegan Diet Study at Massey University. A larger study that assessed a range of health indicators in this sample population. The focus of this study was determining participant's iron intake from the diet and iron status, and the investigation of other nutrition, health and lifestyle factors that influence iron status in this sample. The study was approved by the Health & Disability Ethics Committee (HDEC). Written informed consent was obtained from all participants.

3.2.1 Participants and Recruitment

Men and women aged ≥18 years, who had followed a vegan diet for at least 2 years before participation were recruited from Auckland, New Zealand from August 2022 to March 2023. Recruitment was carried out through vegan Facebook groups and other social media platforms, through special interest groups, posters displayed around Auckland businesses and through word of mouth (see Appendix B). Participants did not have to live in Auckland but had to attend one in-person visit to the Massey University Albany Campus. Participants that were pregnant, had inflammatory conditions, bowel disease and known haemochromatosis were excluded from the study.

An appropriate sample size of 200 was calculated to achieve statistical power to address the range health outcomes assessed in the Vegan Diet Study.

3.2.2 Procedures and Methodology

Each participant attended one in-person visit to Human Nutrition Unit at Massey University Albany Campus for data collection. During the appointment the participants completed questionnaires in which lifestyle and demographic information was gathered (see Appendix E). Following the questionnaires, anthropometrics and a venous blood sample were collected. Participants were then asked to complete a four-day food record (4D-FR) proceeding the in-person visit (see Appendix F). All data collection procedures were carried out by trained study personnel using standard operating procedures (see Appendices D&E).

3.2.3 Dietary Analysis and Questionnaires

The participants completed a 4D-FR of their usual diet following the in-person visit, which involved recording everything they ate or drank over four consecutive days (three weekdays and one weekend day) (see Appendix F). Instructions on how to complete this were provided at the in-person visit and a food record example with instructions was provided for participants to take home. They were asked to include pictures or any relevant extra information, such as recipes for home-cooked meals and snacks, with their diaries. Once completed and returned, the diaries were checked and if any incorrect or missing information was suspected participants were contacted and sent a follow up sheet requesting the information.

Table 3.1 shows the questions relevant to iron status contained in the questionnaires (see Appendix F).

Table 3.1 *Questions in Questionnaire Relevant to Iron Status*

Duration of following a vegan diet
Use of iron supplement, or multivitamin/other supplement that contains iron, and asked for the brand they use and frequency of use
Diagnoses with acute or chronic illness
Previous diagnosis with iron deficiency
Non-menstrual blood loss: nose bleeds, blood donations and any other blood loss
Past iron infusions or blood transfusions
Gender and ethnicity
Physical activity levels
Questions specific to women:
Menstruation status, menstrual bleeding length and time between menstrual bleeding
Hormonal contraceptive use
If they had been pregnant in the last year and if there was significant blood lost, that they knew of

3.2.4 Body Composition

Body composition was measured using body mass index (BMI) in kg/m^2. Weight was measured using the Inbody 230 scale. Height was measured using the SECA 510 stadiometer. Body fat percentage was determined using the DEXA Hologic QDR series Horizon (Wisconsin, MA, USA).

3.2.5 Blood Analysis

Participants provided a venous blood sample of approximately 20ml. 10µL of blood was added to a haemocue cuvette and used to measure Haemoglobin (Hb) on-site using HemoCue® Hb 201+ System. The remainder of the blood sample was centrifuged, within 2 hours of collection, at 3500rpm for 15 minutes at 4°C using rotor 8178/9. Once centrifuged, 3mL of plasma was aliquoted and stored in the freezer at -80°C until study completion, after which samples were sent to North Shore Hospital for analysis. The biomarkers of interest analysed were serum ferritin, iron, transferrin, transferrin saturation and CRP. ID was defined as SF <30µg/L. Compared to lower SF cut-offs used in prior research (SF <12µg/L to <15µg/L) the cut-off of <30 µg/L is associated with a higher sensitivity to detect ID (Daru et al., 2017) (Soppi, 2018). Iron deficiency anaemia (IDA) was defined as SF <30µg/L and Hb <130g/L for males and <120g/L for females (Pfeiffer & Looker, 2017).

3.2.6 Dietary Analysis

The completed four-day food diaries were entered into FoodWorks (Xyris Software, Australia Pty Ltd) by trained MSc Nutrition and Dietetic students using a code book of assumptions to ensure consistency and accuracy (see Appendix H).

The foods were selected from the NZ FOODFiles 2018 data file, primarily, and the Australian food data files (AusBrands 2019 and AusFoods 2019) for packaged foods not found in NZFOODFiles 2018. When a food item was not available in these data files a new food was manually entered using an existing food file of a similar food available in the data files and overridden with the known nutrients of the new food. Every one in five entries were audited by a research assistant for errors and, if found, were rectified. Finally, the nutrient information was collated and any participant outliers that were identified as consuming high amounts of a particular nutrient/s had their food diaries re-checked to ensure the information entered was correct. Any decisions were made in collaboration with the research team (see Appendix H).

FoodWorks was used to analyse the mean daily intakes of iron, energy, protein and enhancers (Vitamin C) and inhibitors (calcium, dietary fibre, caffeine) of iron absorption (Piskin et al., 2022). Mean intake of iron was assessed excluding supplements, however, supplement intake of iron, vitamin C and calcium was recorded (user or non-user) without quantification. The Nutrient Reference Values (NRV), specifically estimated average requirement (EAR), for Australian and New

Zealand adults was used to assess adequate intake. Inadequate iron intake was defined as <100% of the EAR.

3.2.7 Statistical Analysis

Statistical analysis was carried out using IBM SPSS Statistics (version 27.0; SPSS Inc., Chicago, IL). The data was not checked for normality because according to the central limit theorem (CLM) the sampling distribution of the mean will be normally distributed provided the sample size is big enough, sample sizes ≥30 are considered sufficient (Field, 2013). Therefore, data is presented as mean ± standard deviation. Categorical data is presented as number and percentage for each group.

Comparisons were made using independent t-tests (for parametric continuous data) or chi square test (for categorical data). For chi-squared test, the expected count for each cell was ≥ 5, and all groups were independent. If the expected count was <5, the Fisher's Exact Test was used. For all tests, a p-value of ≤0.05 was considered statistically significant.

3.3 Results

3.3.1 Participant Characteristics

The study recruited 212 participants; however, several participants were excluded due to being unable to provide blood (n=9), having haemochromatosis (n=2), Crohn's disease (n=2) and leukaemia (n=1). Another three female participants were excluded due to unknown cause of low Hb concentrations (<120g/L); their ferritin, CRP and B12 concentrations were normal. This resulted in 195 participants, aged from 19 to 75 years old, being included in the analysis. A further 16 participants were excluded from the dietary intake and supplement analysis because they did not return their food diaries.

Table 3.2 displays the participant characteristics and health and lifestyle information for total participants and compares males and females. Most participants were female (n=142; 72.8%) and the mean age of participants was 39.8 ±12.4 years, with no significant difference in the age between males (41.2 ±12.0) and females (39.3 ±12.6), p=0.341. Most participants were NZ European (n=167; 85.6%) and educated to bachelor's degree level or higher (69.2%).

BMI did not significantly differ between males and females; however, mean body fat percentage was significantly different between males (22.8 ±5.1%) and females (33.1 ±6.0%) (p≤0.001).

Most of the participants had been following a vegan diet for 2 to 4 years (40.5%) or 5 to 10 years (42.6%) and there was no significant difference between males and females.

Table 3.2 *Participant Demographics, Health, and Lifestyle Information*

Characteristics		Total (195)	Male (n=52)	Female (n=143)	p-value[+]
Age (years) (mean ± SD)		39.8 ± 12.4	41.2 ± 12.0	39.3 ± 12.6	0.341
BMI (kg/m^2) (mean±SD)		23.9 ± 3.1	24.5 ± 3.0	23.7 ± 3.2	0.144
Body Fat % (mean±SD)		30.4 ± 7.36	22.8 ± 5.1	33.1 ± 6.0	**≤0.001**
Ethnicity (n (%))	*NZ European*	167 (85.6)	45 (23.1)	122 (62.6)	0.829
	Other	28 (14.4)	7 (3.6)	21 (10.8)	
Education (n (%))	*≤ High School*	18 (9.2)	7 (3.6)	11 (5.6)	**0.019**
	Diploma/Certificate	42 (21.5)	17 (8.7)	25 (12.8)	
	≥ Bachelor's degree	135 (69.2)	28 (14.4)	107 (54.9)	
Years following a vegan diet (n (%))	*2 to 4 years*	79 (40.5)	20 (10.3)	59 (30.3)	0.576
	5 to 10 years	83 (42.6)	25 (12.8)	58 (29.7)	
	More than 10 years	33 (16.9)	7 (3.6)	26 (13.3)	
Physical activity (n (%))*	*Low*	32 (17.0)	8 (4.2)	24 (12.6)	0.164
	Moderate	147 (76.9)	35 (18.3)	112 (58.6)	
	High	12 (6.2)	6 (3.1)	6 (3.1)	
Chronic illness (n (%))	*Yes*	46 (23.6)	10 (5.1)	36 (18.5)	0.449
	No	149 (76.4)	42 (21.5)	107 (54.9)	
Smoking Status (n (%))	*Yes*	10 (5.1)	3 (1.5)	7 (3.6)	0.728
	No	185 ± 94.9	49 (25.1)	136 (69.7)	

+ p-value to test differences between means and frequencies between groups as assessed by independent t-tests (for parametric continuous data) or chi square test (for categorical data)
*only 191 participants provided PAL information

3.3.1 Iron Status

Table 3.3 shows the mean iron status of all participants. No participants had iron deficiency anaemia (Hb <130g/L in males and <120g/L in females and SF <30µg/L). The prevalence of SF <20µg/L and iron SF <30µg/L was 22.1% and 25.2%, respectively. Low serum ferritin prevalence rates were higher in females compared to males; 2.1% of males SF <20µg/L and 2.1% had SF <30µg/L compared to 20.0% and 23.1%, respectively, of females.

All iron biomarkers were significantly different in males and females. The mean SF concentration of participants was 41.9 ± 35.2µg/L. SF concentrations in females were 32.9 ±26.2 µg/L and 65.9 ±44.2µg/L in males, p=≤0.001.

Table 3.3 *Biomarkers of Iron Status in Total Participants and Males Compared to Females*

	Total	Male	Female	p-value[+]
Iron Deficiency Anaemia[a] (n (%))	0 (0.0)	0 (0.0)	0 (0.0)	
SF <20µg/L (n (%))	43 (22.1)	4 (2.1)	39 (20.0)	**≤0.001**
SF 20-30µg/L (n (%))	49 (25.2)	4 (2.1)	45 (23.1)	
SF ≥30µg/L (n (%))	103 (52.9)	44 (22.6)	59 (30.3)	
Serum Iron (umol/L) (mean ± SD)	15.1 ± 6.5	16.5 ± 6.5	14.6 ± 6.4	0.070
Serum Iron Binding (umol/L) (mean ± SD)	57.0 ± 11.5	53.3 ± 8.2	58.4 ± 12.3	**0.006**
Serum Ferritin (ug/L) (mean ± SD)	41.9 ± 35.2	66.8 ± 44.1	32.8 ± 26.2	**≤0.001**
Transferrin Saturation (%) (mean ± SD)	26.9 ± 11.0	30.7 ± 10.5	25.5 ± 10.8	**0.002**
Haemoglobin (g/L) (mean ± SD)	154 ± 15	163.1 ± 13.9	151.6 ± 14.8	**≤0.001**

a Haemoglobin <130g/L (men) or <120g/L (women) and serum ferritin <30µg/L
SF = serum ferritin
+ p-value to test differences between means and frequencies between groups as assessed by independent t-tests (for parametric continuous data)

3.3.2 Nutrient Intake from Food and Supplements

Table 3.4 shows participant's nutrient and supplement intake. Males had a significantly higher intake of most nutrients except for vitamin C and caffeine. Total mean iron intake was 18.6mg/day (±6.0mg), males consumed 22.3mg/day (±6.4mg) compared to 17.2mg/day (±5.2mg) in females, p=≤0.001. From food alone, the mean intake in females did not meet the recommended dietary intakes (RDI) for iron (RDI 18mg/day) and calcium (RDI 1,000-1,300mg/day). The RDI and average intake (AI) for vitamin C and dietary fibre was exceeded in both males and females. In total, 21.8% of participants reported taking iron supplements or multivitamins containing iron.

The final section shows the number of participants meeting EAR and RDI for iron. Most participants met the RDI and EAR. However, 62.5% of menstruating females did not meet the RDI for iron intake (see Appendix I, table A for supplementary methods).

Table 3.4 *Nutrient Intake from Food and Supplements in Total Sample and Males Compared to Females*

Nutrient		Total (n=179)	Male (n=48)	Female (n=131)	p-value+
Food Intake (mean ± SD):					
Energy (kJ/day)		9060 ± 2523	11310 ± 2747	8225 ± 1852	**≤0.001**
Protein (g/day)		78.0 ± 27.6	97.9 ± 34.0	69.3 ± 20.2	**≤0.001**
Dietary Fibre (g/day)		46.5 ± 15.3	55.1 ± 18.5	43.4 ± 12.7	**≤0.001**
Iron (mg/day)		18.6 ± 6.0	22.3 ± 6.4	17.2 ± 5.2	**≤0.001**
Calcium (mg/day)		925 ± 354	1058 ± 380	876 ± 332	0.002
Vitamin C (mg/day)		145 ± 99	149 ± 79	143 ± 105	0.708
Caffeine (mg/day)		182 ± 125	173 ± 115	185 ± 129	0.570
Supplement Intake (n (%)):					
Iron Supplement User	Yes	15 (8.4)	1 (0.6)	14 (7.8)	0.074
	No	164 (91.6)	47 (26.3)	117 (65.4)	
Multivitamin Containing Iron	Yes	21 (11.7)	2 (1.1)	19 (10.6)	0.068
	No	158 (88.3)	46 (25.7)	112 (62.6)	
Vitamin C Supplement	Yes	33 (18.4)	6 (3.4)	27 (15.1)	0.278
	No	146 (81.6)	42 (23.5)	104 (58.1)	
Calcium Supplement	Yes	28 (15.6)	4 (2.2)	24 (13.4)	0.112
	No	151 (84.4)	44 (24.6)	107 (59.8)	
Recommended Dietary Intake (RDI) & Estimated Average Requirements (EAR) (n)					
18mg (RDI)	<	-	-	77	-
	≥			54	
8mg (RDI & EAR)	<	3	0	3	-
	≥	176	48	128	
6mg (EAR)	<	-	0	-	-
	≥		48		
5mg (EAR)	<	-	-	1	-
	≥			130	

+ p-value to test differences between means and frequencies between groups as assessed by independent t-tests (for parametric continuous data) or chi square test (for categorical data)

3.3.3 Iron Status and Risk Factors

Table 3.5 presents data on iron status and different risk factors for ID. The iron status groups were condensed to ID (SF <30µg/L) and iron sufficient (SF ≥30µg/L)). Significantly more females were iron deficient (58.7% of females compared to 15.4% of males; (p≤0.001) because of this the risk factors were assessed separately in males and females with details presented in tables 3.6 and 3.7. The age of ID participants was significantly lower than those who were iron sufficient (36.5 ± 11.5 compared to 42.7 ± 12.6; (p≤0.001). Amongst ID participants, previous diagnoses of iron deficiency (p=0.001) and donating blood within the last 12 months was higher in the ID participants (p=0.005).

There was a significant difference in energy intake between the iron status groups (p=0.030) but there was no significant difference with intake of protein, dietary fibre, iron, calcium, vitamin C and caffeine. There was also no significant difference in supplement use between the two groups.

Table 3.5 *Comparison of risk Factors in Total Iron Deficient and Iron Sufficient Participants*

Characteristics:		Deficient (n=92)[a]	Sufficient (n=103)[b]	p-value[+]
Sex (n (%))	Male	8 (4.1)	44 (22.6)	≤0.001
	Female	84 (43.1)	59 (30.3)	
Age (years) (mean ± SD)		36.5 ± 11.5	42.7 ± 12.6	≤0.001
BMI (kg/m2) (mean ± SD)		23.5 ± 2.9	24.3 ± 3.3	0.069
Body Fat % (mean ± SD)		31.5 ± 6.5	29.5 ± 8.0	0.055
Ethnicity (n(%))	*NZ European*	78 (40)	89 (45.6)	0.747
	Other	14 (7.2)	14 (7.2)	
Education (n(%))	*≤ High School*	7 (3.6)	11 (5.6)	0.567
	Certificate/Diploma	18 (9.2)	24 (12.3)	
	≥ Bachelor's	67 (34.4)	68 (34.9)	
Years following a vegan diet (n (%))	*2 to 4 years*	38 (19.5)	41 (21)	0.778
	5 to 10 years	37 (19)	46 (23.6)	
	More than 10 years	17 (8.7)	16 (8.2)	
Previous diagnosis with iron deficiency (n (%))	*Yes*	43 (22.1)	23 (11.8)	≤0.001
	No	49 (25.1)	80 (41)	
Previous iron infusion (n (%))	*Yes*	87 (44.6)	101 (51.8)	0.191
	No	5 (2.6)	2 (1.0)	
Nose Bleeds (n (%))	*Yes*	3 (1.5)	7 (3.6)	0.264
	No	89 (45.6)	96 (49.2)	
Donated Blood within the last 12 months (n (%))	*Yes*	13 (6.7)	3 (1.5)	*0.004*
	No	79 (40.5)	100 (51.3)	
Physical activity (n (%))	*Low*	17 (8.9)	15 (7.9)	0.769
	Moderate	68 (35.6)	79 (41.4)	
	High	6 (3.1)	6 (3.1)	
Chronic illness (n (%))	*Yes*	23 (11.8)	23 (11.8)	0.661
	No	69 (35.4)	80 (40.0)	
Smoking Status (n (%))	*Yes*	5 (2.6)	5 (2.6)	0.854
	No	87 (44.6)	98 (50.3)	
Food Intake (mean ± SD):				
Energy (kJ/day)		8610 ± 2452	9432 ± 2532	0.030
Protein (g/day)		74.5 ± 27.7	79.1 ± 27.5	0.266
Dietary Fibre (g/day)		45.3 ± 18.1	47.5 ± 12.6	0.348
Iron (mg/day)		18.0 ± 6.7	19.1 ± 5.4	0.253
Calcium (mg/day)		935 ± 408	916 ± 304	0.718
Vitamin C (mg/day)		143 ± 97	146 ± 100	0.885
Caffeine (mg/day)		178 ± 128	185 ± 124	0.712
Supplement Intake (n (%)):				
Iron Supplement User	Yes	10 (5.6)	5 (2.8)	0.082
	No	71 (39.7)	93 (52.0)	
Multivitamin Containing Iron	Yes	13 (7.3)	8 (4.5)	0.103
	No	68 (38.0)	90 (50.3)	
Vitamin C Supplement	Yes	18 (10.1)	15 (8.4)	0.235
	No	63 (35.2)	83 (46.4)	
Calcium Supplement	Yes	16 (8.9)	12 (6.7)	0.169
	No	65 (36.3)	86 (48.0)	

+ p-value to test differences between means and frequencies between groups as assessed by independent t-tests (for parametric continuous data) or chi square test (for categorical data).
a serum ferritin <30µg/L
b serum ferritin ≥30µg/L

3.3.4 Determinants of Iron Status in Females

Table 3.6 shows that ID females were significantly younger that iron sufficient females (p≤0.001). The number of ID females (9.1%) that had donated blood within the last 12 months was also significantly higher than iron sufficient females (1.4%) (p=0.025). There were 70 (49.3%) ID females still menstruating, and this was significantly higher than that of iron sufficient females (26.1%) (p=0.010). The prevalence of ID in females still menstruating was 65.4%. There were no significant differences in nutrient intakes between ID and iron sufficient women.

Table 3.6 *Comparison of Variables in Female Iron Deficient and Iron Sufficient Participants*

Female Characteristics:		Deficient (n=83)[b]	Sufficient (n=59)[c]	p-value[+]
Age (years) (mean±SD)		36.1 ± 11.4	43.8 ± 12.9	***≤0.001***
BMI (kg/m2) (mean±SD)		23.4 ± 2.9	24.2 ± 3.4	0.100
Body Fat % (mean±SD)		32.5 ± 5.7	34.1 ± 6.4	0.115
Ethnicity (n(%))	*NZ European*	72 (50.3)	50 (35.0)	>0.999
	Other	12 (8.4)	9 (15.3)	
Education	*≤ High School*	7 (4.9)	4 (2.8)	0.916
	Certificate/Diploma	14 (9.8)	11 (7.7)	
	≥ Bachelor's	63 (44.1)	44 (30.8)	
Years following a vegan diet (n (%))	*2 to 4 years*	36 (25.2)	23 (16.1)	0.901
	5 to 10 years	33 (23.1)	25 (17.5)	
	More than 10 years	15 (10.5)	11 (7.7)	
Previous diagnosis with iron deficiency (n (%))	*Yes*	42 (29.4)	22 (15.4)	0.172
	No	42 (29.4)	37 (25.9)	
Previous iron infusion (n (%))	*Yes*	79 (55.2)	57 (39.9)	0.700
	No	5 (3.5)	2 (1.4)	
Nose Bleeds (n (%))	*Yes*	2 (1.4)	5 (3.5)	0.125
	No	82 (57.3)	54 (37.8)	
Donated Blood within the last 12 months (n (%))	*Yes*	13 (9.1)	2 (1.4)	***0.025***
	No	71 (49.7)	57 (39.9)	
Physical activity (n (%))	*Low*	15 (10.6)	9 (6.3)	0.442
	Moderate	63 (44.4)	49 (34.5)	
	High	5 (3.5)	1 (0.7)	
Chronic illness (n (%))	*Yes*	22 (15.4)	14 (9.8)	0.845
	No	62 (43.4)	45 (31.5)	
Smoking Status (n (%))	*Yes*	5 (3.5)	2 (1.4)	0.700
	No	79 (55.2)	57 (39.9)	
Food Intake (mean±SD)*:				
Energy (kJ/day)		8,100 ± 1,581	8,416 ± 2,165	0.336
Protein (g/day)		69.7 ± 21.8	68.8 ± 17.8	0.808
Dietary Fibre (g/day)		42.4 ± 13.4	44.6 ± 11.7	0.332
Iron (mg/day)		17.1 ± 5.7	17.4 ± 4.7	0.722
Calcium (mg/day)		892 ± 374	853 ± 266	0.509
Vitamin C (mg/day)		137 ± 94	152 ± 119	0.422
Caffeine (mg/day)		178 ± 130	194 ± 129	0.484
Supplement Intake (n (%)):				
Iron Supplement User	Yes	10 (7.6)	4 (3.1)	0.392
	No	65 (49.6)	52 (39.7)	
Multivitamin Containing Iron	Yes	12 (9.2)	7 (5.3)	0.625
	No	63 (48.1)	49 (37.4)	
Vitamin C Supplement	Yes	16 (12.2)	11 (8.4)	>0.999
	No	59 (45)	45 (34.4)	
Calcium Supplement	Yes	15 (11.5)	9 (6.9)	0.651
	No	60 (45.8)	47 (35.9)	
Reproductive Information (n (%)):				

Menstrual Status (n=142)	*Never menstruated*	0 (0.0)	1 (0.7)	0.010
	Menstruating	70 (49.3)	37 (26.1)	
	Menopause	5 (3.5)	5 (3.5)	
	Postmenopausal	8 (5.6)	16 (11.3)	
Menstruation Frequency (n=117)	*Regular*	50 (42.7)	31 (26.5)	0.532
	Irregular	25 (21.4)	11 (9.4)	
Pregnant within the last 12 months (n=117)	*Yes*	3 (2.6)	1 (0.9)	>0.999
	No	72 (61.5)	41 (35.0)	
Using Hormonal Contraceptives (n=142)	*Yes*	16 (11.3)	13 (9.2)	0.567
	No	64 (45.1)	42 (29.6)	
	Not specified	3 (2.1)	4 (2.8)	

+ p-value to test differences between means and frequencies between groups as assessed by independent t-tests (for parametric continuous data) or chi square test (for categorical data).
a serum ferritin <30µg/L
b serum ferritin ≥30µg/L
*n=133 (n=9 did not return food records)

3.3.5 Determinants of Iron Status in Males

Only nutrient intake was significantly different in ID compared to iron sufficient men. Iron deficient men had higher energy (p≤0.001), protein (p=0.004), dietary fibre (p≤0.001), iron (p=0.001), calcium (p=0.003) and vitamin C (p=0.006) but no significant difference in caffeine intakes.

Table 3.7 *Comparison of Variables in Male Iron Deficient and Iron Sufficient Participants*

Male Characteristics:		Deficient (n=8)[b]	Sufficient (n=44)[c]	p-value[+]
Age (years) (mean ± SD)		40.8 ± 11.4	41.3 ± 12.2	0.922
BMI (kg/m2) (mean ± SD)		24.9 ± 2.1	24.4 ± 3.1	0.683
Body Fat % (mean ± SD)		21.0 ± 4.6	23.2 ± 5.2	0.251
Ethnicity (n(%))	*NZ European*	6 (11.5)	39 (75)	0.291
	Other	2 (3.8)	5 (9.6)	
Education (n(%))	*≤ High School*	0 (0.0)	7 (13.5)	0.439
	Certificate/Diploma	4 (7.7)	24 (46.2)	
	≥ Bachelor's	4 (7.7)	13 (25)	
Years following a vegan diet (n (%))	*2 to 4 years*	2 (3.8)	18 (34.6)	0.433
	5 to 10 years	4 (7.7)	21 (40.4)	
	More than 10 years	2 (3.8)	5 (9.6)	
Previous diagnosis with iron deficiency (n (%))	*Yes*	1 (1.9)	1 (1.9)	0.287
	No	7 (13.5)	43 (82.7)	
Previous iron infusion (n (%))	*Yes*	0 (0.0)	0 (0.0)	
	No	8 (15.4)	44 (84.6)	
Nose Bleeds (n (%))	*Yes*	1 (1.9)	2 (3.8)	0.401
	No	7 (13.5)	42 (80.0)	
Donated Blood within the last 12 months (n (%))	*Yes*	0 (0.0)	1 (1.9)	>0.999
	No	8 (15.4)	43 (82.7)	
Physical activity (n (%))	*Low*	2 (4.1)	6 (12.2)	0.827
	Moderate	5 (10.2)	30 (61.2)	
	High	1 (2.0)	5 (10.2)	
Chronic illness (n (%))	*Yes*	1 (1.9)	9 (17.3)	>0.999
	No	7 (13.5)	35 (67.3)	
Smoking Status (n (%))	*Yes*	0 (0.0)	3 (5.8)	>0.999
	No	8 (15.4)	41 (78.8)	
Food Intake*:		n=4	n=41	
Energy (kJ/day) (mean ± SD)		14,987 ± 2,549	10,786 ± 2,366	≤0.001
Protein (g/day) (mean ± SD)		133.9 ± 25.2	92.8 ± 32.1	0.004
Dietary Fibre (g/day) (mean ± SD)		81.4 ± 29.7	51.3 ± 12.9	≤0.001

Iron (mg/day) (mean ± SD)		29.9 ± 7.4	21.3 ± 5.5	**0.001**
Calcium (mg/day) (mean ± SD)		1,472 ± 464	999 ± 334	**0.003**
Vitamin C (mg/day) (mean ± SD)		230 ± 110	138 ± 68	**0.006**
Caffeine (mg/day) (mean ± SD)		175 ± 110	173 ± 117	0.957
Supplement Intake:				
Iron Supplement User (n (%))	**Yes**	0 (0.0)	1 (2.1)	>0.999
	No	6 (12.5)	41 (85.4)	
Multivitamin Containing Iron (n (%))	**Yes**	1 (2.1)	1 (2.1)	0.237
	No	5 (10.4)	41 (85.4)	
Vitamin C Supplement (n (%))	**Yes**	2 (4.2)	4 (8.3)	0.157
	No	4 (8.3)	38 (79.2)	
Calcium Supplement (n (%))	**Yes**	1 (2.1)	3 (6.3)	0.425
	No	5 (10.4)	39 (81.3)	

+ p-value to test differences between means and frequencies between groups as assessed by independent t-tests (for parametric continuous data) or chi square test (for categorical data).
a serum ferritin <30µg/L
b serum ferritin ≥30µg/L
*n=47 (n=5 did not return food records)

3.4 Discussion

Overall, nearly half of participants were ID. Rates of ID were significantly higher in females compared to males. Iron intake was high overall, especially in men. However, a significant difference between iron intake and intake of iron inhibitors and enhancers was only found between ID and iron sufficient males, not females. Whereas a significant difference in non-dietary determinants of ID was found in females and included younger age, blood donation within the last 12 months and still menstruating.

3.4.1 Prevalence of Iron Deficiency Compared to Other Groups

The participants were initially categorised into four iron status groups to allow for comparison with existing research. There were no cases of IDA amongst the participants which contrasts with previous research completed in non-vegan specific research within New Zealand. The 2008/2009 New Zealand (NZ) Nutrition Survey found that 2% of participants were IDA (University of Otago & Ministry of Health, 2011). The only vegan-specific research that reported IDA rates in vegans found prevalence was 4% in young women (YW) (18-50) and 4% in old women (OW) (50+) (Waldmann et al., 2004).

Overall, 47.3% of vegans in this study were ID using the SF cut-off of <30µg/L, this was significantly higher in females (58.7%) compared to males (15.4%). Waldmann at al. (2004) also found very high rates of ID in females, 52% of YW were ID and 20% of OW, 40%. However, in this study they used a much lower cut-off (<12µg/L) to define ID, therefore prevalence rates may have been much higher had they used the higher cut-off <30µg/L. Weikert et al. (2020) used a slightly higher cut-off (<15µg/L for females and <30µg/L for males) and combined males and females. They found a much lower prevalence of ID and no significant difference between vegan (11.1%) and omnivores (8.3%). In addition, two Australian studies found 11.1% and 20%, respectively, adult male vegans had low iron stores (<12µg/L) (Li et al., 2000; Wilson & Ball, 1999). Comparing our research to other vegan research is difficult due to the different SF cut-offs used to define ID and different categorisation of groups which may be a consideration for future research.

Comparing ID prevalence to other NZ research, the NZ Nutrition Survey (NNS) (which includes omnivores) found much lower rates of ID (4.2% of the total sample, 8.4% of females and 1.5% of males), however, they also used the lower SF cut-off of <12µg/L (University of Otago & Ministry of Health, 2011). Only two other studies in premenopausal women were found to compare ID prevalence in NZ sample populations. One study, which included omnivores, found that 18.7% pre-menopausal female participants had SF <20µg/L (Beck et al., 2014). This was much lower than our finding of 26.8% with SF <20µg/L in total females and even lower (33.6%) when only pre-menopausal women were included (see Appendix I, Table B for supplementary results). The second study found a high number (55.8%) of female participants had SF <30µg/L, similar to the findings in our study (Lim et

al. 2020). Interestingly, 34.8% of participants in the study by Lim et al. (2020) were classed as vegetarian and they also had a high number of South Asian participants, who may have a higher incidence of ID (Beck et al., 2014). When compared to the vegan female participants in our study, who were mostly NZ European (85.3%), and when only pre-menopausal females were considered ID prevalence was much higher in the current study (65.4%). Unlike the current study, Lim et al. (2020) did exclude blood donors, however, there is the possibility that had these individuals been included the prevalence rate of ID may have been similar to pre-menopausal females in the NZ vegan sample or higher.

Serum ferritin of the vegans in this study is significantly lower compared to the NNS sample. Mean SF concentrations in the NZ vegan sample was 65.9µg/L for males and 32.9µg/L for females. The NNS found mean SF concentrations were 177µg/L for males and 80µg/L females (University of Otago & Ministry of Health, 2011). Existing research comparing SF concentrations of vegans to other diet groups found no significant difference between groups (Elorinne et al., 2016; Gallego-Narbón et al., 2019; Henjum et al., 2021; Obeid et al., 2002; Schüpbach et al., 2017; Waldmann et al., 2004; Weikert et al., 2020). Only two vegan studies found lower SF concentrations in vegans, however, participant numbers were low (n=18 & n=10, respectively) (Li et al., 2000; Wilson & Ball, 1999). Of note, in most of the studies the number of vegan participants were low and compared to other diet groups with much higher sample sizes, which is likely to have impacted the statistical power and limited the ability to observe a true effect (Zhao et al., 2021).

3.4.2 Dietary Iron Intake as a Determinant of Iron Status

Mean iron intake in the NZ vegan sample was 18.6mg/day overall and significantly different in males (22.3mg/day) compared to females (17.2mg/day). The NNS found significantly lower mean iron intake; 11.9mg/day in total, 13.8mg/day in males and 10.1mg/day in females (University of Otago & Ministry of Health, 2011). Given the NNS sample had significantly higher SF concentrations yet lower intakes of dietary iron, this finding may add to the evidence that although iron intake may be high in vegans, it is less bioavailable.

Most research investigating iron intake (not including supplements) in vegans found that iron intake was above the RDI for men and women (Allès et al., 2017; Clarys et al., 2014; Elorinne et al., 2016; Li et al., 2020; Schüpbach et al., 2017; Waldmann et al., 2004). Mean iron intakes in the NZ vegan sample well exceeded EAR for males and females, only four females did not meet the EAR. Males also exceeded mean intakes for RDI, however, mean iron intake in women was 17.2mg/day, which does not meet the RDI for menstruating women. Two other studies reported iron intakes below the RDI within vegan participants (Davey et al., 2003; Kristensen et al., 2015). Kristensen et al. (2015) found

average intake was 13.5mg/day in males and females. However, lower intakes may be explain by the fact that fortified food was banned in Denmark until 2003 and is still not widely available at the time of the study (Buch-Weeke, 2014). Davey et al. (2003) discussed the limitations of the FFQ underestimating energy intake which was used to assess iron intake in the second study on vegan participants. They found that vegetarians and vegans reported lower energy intakes and this may be due to the uniform portition sizes used for men and women, which may be especially pertinant to these groups because they may consume larger portions of carbohydrate foods. Thus most reported nutrients, inclusive of iron may be lower (M: 15.3mg/day; F: 14.1mg/day).

Overall, iron intakes are not too dissimilar to previous research on iron intake in vegans. However, previous vegan research has also found that iron intakes are higher in vegans compared to omnivores (Allès et al., 2017; Clarys et al., 2014; García-Morant et al., 2020; Kristensen et al., 2015; Nebl et al., 2019; Schüpbach et al., 2017; Weikert et al., 2020). Similarly, iron intakes in the NZ vegan sample compared to the NNS findings were significantly higher. The NNS used a single 24-hour diet recall (24HR), whereas this study used a four-day FR. The FR is recognised as a more comprehensive method for estimating energy, and most nutrients intakes, compared to other methodologies and accounts for day-to-day variation in intake, which a single 24HR does not (Bingham et al., 1994; Prentice et al., 2011). Therefore this difference between the NZ vegan sample of the current study and NNS participants may add to the body of evidence that iron intake tends to be higher in vegans compared to omnivores.

Iron intake and intake of iron enhancers and inhibitors were significantly different in ID compared to iron sufficient groups in males but not in females. Interestingly, ID males had a significantly higher iron intake (28.9mg/day) compared to iron sufficient males (21.3mg/day), with intakes that were well above the RDI in both groups (Australian Government Department of Health and Ageing & Ministry of Health, 2006). This somewhat contradictory finding may be explained by ID males significantly higher dietary fibre intake (81.4mg/day)*****. This high fibre intake was checked for accuracy during food diary review and analysis. There is no upper limit set for dietary fibre intake and the NRV cite a systematic review stating that there is no convincing evidence that dietary fibre inhibits mineral absorption in humans (Australian Government Department of Health and Ageing & Ministry of Health, 2006) (Gordon, Stoops, Ratliff, 1995). Contrarily, some research has demonstrated an inhibitory effect on iron absorption as a result of high fibre intake (Cook et al., 1983) (Péneau et al., 2008). However, the effect may be from other compounds known to inhibit iron absorption which are then found within the dietary fibre; such as phytates, polyphenols and oxalates (Gupta et al., 2006; Hallberg et al., 1989; Hurrell et al., 1999; Marie Minihane & Rimbach, 2002). Iron deficient males also had significantly higher calcium intake compared to iron sufficient males (1,472mg/day compared to 999mg/day),

another suspected iron absorption inhibitor (Abioye et al., 2021; Hallberg et al., 1992). It is also important to note that only eight male participants were ID and six of those returned food diaries. While interesting the low statistical power undermines the reliability of this finding.

Iron sufficient females, on average, were older and contained more postmenopausal women, whose RDI for iron is half that of pre-menopausal women because they are no longer menstruating (Australian Government Department of Health and Ageing & Ministry of Health, 2006). Weikert et al. (2020) also found no significant difference in the iron intakes between ≤50 years (young women) compared to >50 years (old women) and significantly more young women were ID.

***** The average dietary fibre intakes of all 6 of the 8 ID male participants that returned food diaries were well above recommendations (47g/day to 82g/day), however, one participant was consuming an average of 133.9g/day. All six food diaries were re-checked for accuracy. It was observed that they were consuming large servings of high fibre foods such as oats, pasta, dried fruit and nuts, and legumes.

3.4.3 Other Determinants of Iron Status

Overall, the potential risk factors of ID were sex (being female), younger age, previous diagnosis of ID and blood donation within the last 12 months. Due to the high proportion of females (73.3%) recruited these potential risk factors are likely due to the impact of females. When potential risk factors of ID in males were looked at separately, only nutrient intake was significantly different in the iron status groups.

Menstrual status (still menstruating) also became significant when potential risk factors of ID in females were investigated separately. Females are at higher risk of ID due to menstrual blood losses (Mansour et al., 2021). Most of the NZ vegan female participants were still menstruating (75.4%). Age was significantly lower in ID females (36.1 years) compared to iron sufficient females (43.8 years) which may be explained partly by menstruation status. Menopause occurs around the age of 50 and the iron sufficient group contained more postmenopausal females (27.1% compared to 9.6% in ID females) (Gold, 2011). The RDI for iron in postmenopausal women is half that of pre-menopausal women because they are no longer menstruating (Australian Government Department of Health and Ageing & Ministry of Health, 2006). Weikert et al. (2020) also found no significant difference in the iron intakes between ≤50 years (young women) compared to >50 years (old women) which is likely a factor that contributes to more young women being diagnosed with ID.

In the NZ vegan sample 13 out of 16 participants that had donated blood within the last 12 months were ID. Blood donation has been identified as a predictor of ID in previous research conducted within and external to NZ (Beck et al., 2014; Cançado et al., 2001; Fillet et al., 2021; Røsvik et al., 2009). In a previous study in NZ the risk of ID increased in those that donated 3-4 whole blood units in

the previous 12 months, with 25.1% of these subjects ID compared to 14.1% overall in blood donors (Badami & Taylor, 2008).

Of those with a previous diagnosis of ID, 65.2% were ID at the time of this study. Beck et al (2014) also reported that a previous diagnosis with ID in NZ pre-menopausal females more than doubles the odds of having low iron compared to those that had not been diagnosed previously.

3.5 Strengths & Limitations

A major strength of this study is that, to the best of our knowledge, it is the first study conducted investigating iron intake and status in a NZ vegan sample. It was a large sample and the information gathered allowed the comparison of iron intake and iron status, as well as investigation on the impact of other known ID risk-factors.

While this study included a large sample of NZ vegans, due to time and budget constraints, it was a convenience sample. A convenience sample may not be representative of the sample's population and is only generalisable to those with similar characteristics (Andrade, 2021) (Jager et al., 2017). The majority of participants were NZ European (85.6%), which means the results are only generalisable to NZ European Vegans and ethnic differences in iron intake and status were not able to be explored. The sample also consisted of a large number of pre-menopausal women (50.5%), which allowed investigation of risk factors for this at-risk groups but also affects generalisability to postmenopausal females and males.

Some participants took iron supplements or multivitamins (17%), which may have provided a significant portion of their iron intake. However, due to the inherent difficulties assessing supplement intake, micronutrients intake was not quantified (Bailey et al., 2019). Although, it is worth noting that in this study no significant difference was found in iron status between iron and multivitamin supplement users and non-users.

Finally it was a cross-sectional study design and causation cannot be implied from the findings (Setia, 2016).

3.6 Conclusion

Rates of ID in the total were high (nearly half the sample and over half of females) in the NZ vegan sample but were difficult to compare to previous research due to the variability of SF cut-offs used to define ID. However, the prevalence of ID in the sample of NZ vegan females, especially pre-menopausal females, was much higher. The NZ vegan sample had significantly lower SF concentrations than in the NNS, indicating the NZ vegan sample have a higher prevalence of ID compared to other NZ diet groups.

In the NZ vegan sample, non-dietary determinants of iron status were younger age and still menstruating, a previous diagnosis of ID and blood donation within the last 12 months, which is consistent with previous research.

Iron intake was high in men and postmenopausal women, but most pre-menopausal women (62.5%) did not meet the RDI for iron intake. However, iron intakes in the NZ vegan sample (male, female and overall) were similar to iron intakes found in other research on vegans and were much higher than intakes of the NNS sample. Age was significantly lower and the number that had donated blood within the last 12 months was significantly higher in ID females compared to iron sufficient females, but not in males. Intake of iron absorption inhibitors (calcium and dietary fibre) were high in the ID NZ vegan male sample, but not in females. However, this finding and should be interpreted with caution due to the low number of ID males. Further research with larger samples is needed to confirm both findings in males and females, but especially males.

In the NZ vegan sample, non-dietary determinants of iron status were younger age and still menstruating, a previous diagnosis of ID and blood donation within the last 12 months, which is consistent with previous research.

Chapter 4: Conclusion & Recommendations

4.0 Achievement of Aims & Objectives

The overall aim of the research was to determine iron status of a NZ vegan sample and measure the impact of a vegan diet and other known ID risk factors on iron status.

The total prevalence of ID was 47.3%. However, comparing ID prevalence in the total sample to other vegan research and research on other diet groups in NZ was difficult due to different SF cut-offs being used to define ID. Comparison were possible in pre-menopausal females, where in our study the prevalence of ID was 64.5% using a cut-off of <30µg/L and 33.6% SF <20µg/L. These rates are much higher than prevalence rates found in previous NZ research in non-vegan pre-menopausal women (Beck et al., 2014) (Lim, Beck, Von Hurst, Rutherfurd-Markwick, & Badenhorst, 2020). Cumulatively these results demonstrate that in NZ and among pre-menopausal females, ID is a prevailing risk. Serum ferritin concentrations were much lower in the NZ vegan sample compared to the NNS, even when males and females were compared separately. These findings are at odds with previous research in vegans that compared prevalence of ID in vegans to non-vegans, where most of the research found no significant difference in iron status between different diet groups (Waldmann et al., 2004) (Henjum et al., 2021) (Gallego-Narbón et al., 2019) (Obeid et al., 2002) (Weikert et al., 2020) (Elorinne et al., 2016) (Schüpbach et al., 2017). However, these studies all had low numbers of vegan participants. Findings indicate that a vegan dietary pattern is likely to increase ID risk in the NZ vegan sample, especially in premenopausal NZ vegan females. Thus, the hypothesis that the vegan diet does not have an impact on iron status is rejected.

Further to this, the first objective was to measure intake of iron and iron enhancers and inhibitors from food. This was achieved by obtaining four-day food diaries (4DFD) from participants, which overall had a high adherence rate (193/212 participants completed the 4DFD). The 4DFD were entered into FoodWorks to establish mean iron intakes. Findings identified that males and postmenopausal females had sufficient iron intakes according to the RDI, however, pre-menopausal women had a mean iron intake slightly below the RDI (with 62.5% not meeting the RDI). The alternative hypothesis that iron intakes in the NZ vegan sample would be high or sufficient (as compared to the RDI of iron) is therefore rejected in this subgroup of the vegan sample. Taken with the finding of a high prevalence of ID in pre-menopausal females, health promotion should focus on education on vegan sources of iron-rich food.

A relationship between high intake of iron inhibitors (dietary fibre and calcium) and ID was found in males but not females. Previous research has demonstrated the effect of inhibitors on iron absorption, however, the inhibitory effect has not been demonstrated in the long term (i.e. shown an impact of iron status biomarkers) (Cook et al., 1983) (Péneau et al., 2008) (Hallberg et al., 1989) (Marie Minihane & Rimbach, 2002) (Hurrell et al., 1999) (Gupta et al., 2006) (Abioye et al., 2021). The finding in the sample of NZ vegan males indicates that intake of iron inhibitors may be a risk factor for ID, however, further research with a larger samples is needed.

Overall iron intake was high, yet ID was highly prevalent. Pre-menopausal females were an exception and did not meet the RDI. However, comparing iron intake and SF concentrations in the NZ vegan male sample to the NNS males found that intake of iron was higher in the NZ vegan sample (22.3mg/day compared to 17.2mg/day) but SF concentrations were lower (65.9µg/L compared to 177µg/L) (Australian Government Department of Health and Ageing & Ministry of Health, 2006). This provides further evidence that iron within a vegan diet may be less bioavailable.

It was hypothesized that non-dietary determinants of ID in the vegan sample would be significant. As explained above, ID rates were higher in younger women, which could be due to menstruation. Previous blood donation was also found to be a predictor of ID, with ID in 13/16 of those that had donated blood within the last 12 months. This was not found in males, however, there was only one male blood donor, and they were iron sufficient. Additionally, significantly more participants that had a previous diagnosis of iron deficiency or were ID at the time of the study compared to iron sufficient participants which is a factor that would have contributed to this non-dietary determinant being identified as a predictor of ID in this cohort.

4.1 Strengths & Limitations

To the best of our knowledge this is the first study to assess iron intake and status in a specifically vegan sample population. Only one other study in Germany investigated iron intake and status in specifically vegans, however, the sample size was much smaller (n=75) and only included females (Waldmann et al., 2004). Another five studies investigated iron intake and status in vegans and compared findings to non-vegan diet groups, again the sample sizes of the vegan cohorts were much smaller than this study (Elorinne et al., 2016; Li et al., 2000; Schüpbach et al., 2017; Weikert et al., 2020; Wilson & Ball, 1999).

We used a higher cut-off of (SF <30µg/L) to classify ID participants. The higher cut-off of is associated with higher sensitivity compared to the lower SF cut-offs (SF <12µg/L to <15µg/L ((Daru et al., 2017) (Soppi, 2018). Therefore, our research likely detected individuals in the early stages of ID which prior

research using lower SF cut-offs may not have. A consequence of this decision, however, was the limited ability to compare our findings of ID prevalence to other research, only one NZ study used the higher SF cut-off (Lim et al., 2020).

A range of biomarkers were collected and used for analysis which helped to ensure accurate interpretation of iron status. When SF was interpreted it was adjusted for inflammation. Ferritin is an acute phase protein and SF is an unreliable marker of iron status if inflammation is present (Cappellini et al., 2020). If participants had elevated CRP (CRP >5mg/L) they were removed from the data set. Thus, not affecting mean SF and providing a more accurate reflection of iron status in this cohort of vegans. Soluble transferrin receptor (sTfR) has a high sensitivity and specificity and may be a good surrogate marker of bone marrow iron stores, however, it was not used due to the high cost as this assay analysis (Koulaouzidis, Said, Cottier, & Saeed, 2009).

The food record (FR) has advantages and disadvantages, as with any diet recall method. The weighted FR has the highest accuracy when compared to urine nitrogen excretion, a marker of protein intake (Bingham et al., 1994). A 12 day weighed FR allows the estimation of an individual's iron intake to within 10% of mean typical intake (Heath et al., 2005). These findings were balanced with the significant burden the FR places on the participant and the significant time it takes to process and analyse the FR. Intake was recorded for 4 days and weighing food was optional to reduce burden and encourage adherence. As such 92% of food diaries were returned from participants. Another limitation of the FR is self-reporting can be biased, and participants may underreport and/or not report less socially undesirable foods (Hebert, Clemow, Pbert, Ockene, & Ockene, 1995). A further limitation is the availability of foods in the FoodWorks databases, especially vegan meat, and dairy substitute foods. Foods had to be substituted with other vegan alternatives or new foods had to be created based off ingredients available in the database. Substitutions or new foods were checked to ensure they closely matched the nutrition information panels (NIP) of the original food item. However, often manufactures are not required to include fortified micronutrients, such as iron, on the NIP, impacting accurate measurement of iron intake. Finally, intake of food groups (e.g. fruits and vegetables; cereals and grains; tea and coffee) known to contain enhancers/inhibitors (such as polyphenols, phytates) were not investigated due to time constraints. Intake of single nutrients (calcium, caffeine, dietary fibre, and vitamin C) were used as a proxy.

The NZ vegan participants are a convenience sample. A convenience sample is one that recruits individuals that are conveniently accessible to the study (Jager, Putnick, & Bornstein, 2017). This strategy of participant selection is often used in research due to time and budget constraints (Tyrer & Heyman, 2016). However, this creates a biased sample that is not necessarily representative of the

sample population and the findings are only generalisable to those in the population that have similar characteristic to the sample studied (Andrade, 2021) (Jager et al., 2017). Although, as stated above, the sample number was larger compared to other vegan studies and is therefore more likely to reflect characteristics of a broader vegan cohort.

The sample of the NZ vegans was mostly NZ European and contained a high number of pre-menopausal females, which impacts the generalisability of the study finding to other vegan populations. However, the high prevalence of pre-menopausal females in the sample allowed comparison of our findings to two other NZ studies that used similar SF cut-offs.

An important limitation was that micronutrient intake from supplements was not quantified, which can be a significant source of micronutrients. There are inherent difficulties with assessing supplement intake and the impact of measurement error is unknown (Bailey et al., 2019). We may have found higher mean intake of iron in the NZ vegan sample if intake from supplements were included.

Finally, the study design was cross-sectional. Participants provided one-time information to assess exposures (iron intake and other risk factors for iron deficiency) and outcomes (iron status). While interesting information is gathered, causation cannot be implied from the findings (Setia, 2016), but results may serve as a basis for future research investigations in this population group.

4.2 Recommendations & Future Directions for Research

Given the high rates of ID in this study, especially in pre-menopausal females, it is evident that this group is high-risk for ID. The following are recommendations for practice and future research.

- Health professionals should screen iron status in vegans, especially female vegans, as they are an at-risk group for ID. Therefore, iron status should be checked regularly.
- Based off the results of this study, previous research in vegans and iron absorption studies there is evidence that iron from a vegan diet is less bioavailable than diets that contain haem iron. The relationship between iron status and intake of iron inhibitors in NZ vegan males was observed. However, this was a finding in a small number of male participants. Further research on the impact of iron inhibitors on iron status in vegans would clarify this relationship and help to identify specific inhibitors to iron within this dietary pattern.
- Further research on a more representative sample of the NZ vegan sample will improve generalisability of study findings to the NZ vegan population.

- There is also limited up-to-date research on the iron intake and status of NZ non-vegans. Further research in these populations will allow comparison and identification of important ID risk-factors for different diet group populations.

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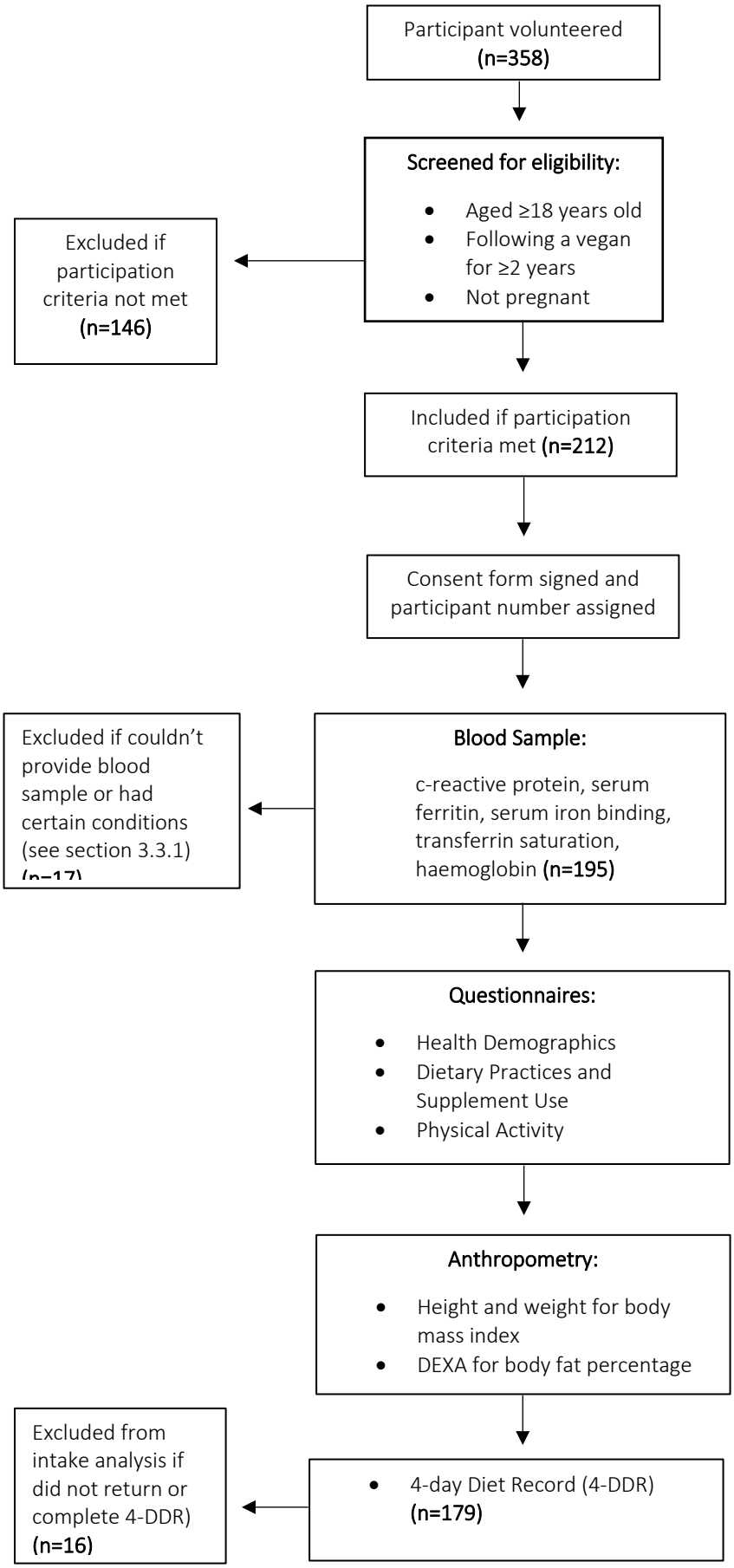
Appendix A: Overview of Study Procedure



Appendix B: Recruitment

Social media posts:

Hi Everyone,

Are you vegan?

Have you been vegan for at least two years?

Do you want to help contribute to a better understanding of the health of a vegan diet?

All genders are welcomed!

Massey University have an exciting new study looking at the effects of a vegan diet on health.

Receive detailed information on your body composition and biochemical nutritional status.

Click here (for those in Auckland only), to express your interest, and one of our research team will be in touch ASAP:

https://massey.au1.qualtrics.com/jfe/form/SV_4PI1ZFiv1CsH2nA

The link to the detailed information sheet is here:

https://www.massey.ac.nz/massey/learning/colleges/college-of-health/school-of-sport-and-exercise/human-nutrition/research/studies/vegan-study/vegan-study-information-sheet.cfm

Poster used for social media and put up at local businesses:

CALLING ALL VEGANS

TAKE PART IN AN EXCITING NEW STUDY

GET YOUR BODY COMPOSITION AND NUTRITION STATUS

EMAIL: VEGANSTUDY@MASSEY.AC.NZ
TEXT OR CALL: 021 220 0092

WWW.MASSEY.AC.NZ/VEGANSTUDY

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Appendix C: Participant Information Sheet

1. Participant Information Sheet

Health and Vegan Diet

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

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

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.

1.1 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 individuals 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?

Individuals 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.

1.2 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.

1.3 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.

1.4 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).

1.5 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.

1.6 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 (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.

1.7 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 D: Standard Operating Procedure for Blood Sample Collection and Analysis

2. **Blood analysis and laboratory where analysis will be performed**

3.1. Vitamin B_{12} at North Shore Hospital Laboratory
3.2. Folate at North Shore Hospital Laboratory
3.3. Iron studies at North Shore Hospital Laboratory
3.4. CRP at North Shore Hospital Laboratory
3.5. Calcium and albumin at North Shore Hospital Laboratory
3.6. vitamin D at North Shore Hospital Laboratory
3.7. Blood spot lipids on site at Massey University
3.8. Blood spot haemoglobin on site at Massey University
3.9. Blood spot HbA1c on site at Massey University
3.10. Dried Blood spot (DBS) fatty acids at OmegaQuant Analytics

3. **Participant identification labelling**

4.1. Give each participant a unique identification number, prefix with the assigned study code number of 88

4. **Tubes selection and preparation**

Refer to the table below for the selection of tubes appropriate for biomarkers of interest.

Serology tube	G1	Vitamin B12	4ml
		Folate	
		Calcium	
		25(OH)D	
		Serum ferritin	
		Albumin	
		CRP	
		Iron studies	
Serology tube	G2	Backup	2ml
	G3	Backup	2 ml
Drop		DBS Fatty acids	30ul
Drop		Hb	10ul
Drop		HbA1c	10ul

Drop		Lipids	10ul

5. Barcode labelling before study day

6.1. Label template is found in:

C:\Users\oarmugri\OneDrive - Massey University\Massey\88_The_Vegan_Study\Samples

6.2. Labels format:

88 – Study ID

1 – Baseline Timepoint

- Subject ID (e.g., 001, 002, ….)

- Sample type and number (G1, G2)

6.3. Examples of label format

Trial #	Timepoint	Participant #	Cryotube	Example label
88	1	001	G1	88 1 001 G11
88	1	005	G2	88 1 005 G2

6. Blood collection, processing, and storage

7.1. Pre-cool the centrifuge to 4 degrees Celsius approximately 20 minutes before usage.

7.2. When vacutainers enter the lab, add to participant's rack, labelled with ID and initials

7.3. Drop (dried blood spot fatty acids, lipids, Hb and HbA1c)

7.3.1. Aliquot whole blood from centre of EDTA tube

7.3.1.1. Add one drop to Afinion cassette for lipids

7.3.1.2. Add one drop to Hemocue cuvette for Hb

7.3.1.3. Add one drop to Afinion cassette for HbA1c

7.2. Venous blood (25(OH)D, B_{12}, folate, ferritin, iron studies, CRP, calcium and albumin)

7.2.1. Centrifuge ALL VACUTAINERS (green) within 2 hours.

- 4 degrees Celsius
- 3500 rpm
- 15 minutes
- Using rotor 8178/9

7.2.2. Upon removal

- Aliquot 2 x 4ml plasma in total (1 x 4 mls from each vacutainer)
- Add to -80 Freezer 2 following freezer health and safety guidelines.

8.1. New Zealand laboratories

8.1.1. Blood samples to be stored on dried ice and flown to the laboratory in the care of New Zealand courier.

8.1.2. Address:

North Shore Laboratory
124 Shakespeare Road,
Takapuna,
Auckland 0620

7. Labelling of storage boxes

Label storage boxes with:

9.1. Study name (Vegan Study) and month & year (e.g., April 2022)
9.2. Type of sample (e.g., G1, G2, fingerpick)
9.3. Box # (number boxes in consecutive order); Box A (capacity approx. 100), B, etc.

8. Logbook (Record keeping)

10.1. Keep two copies of electronic excel record of lab form, one on a portable media and the other on secure network.

10.2. Keep a paper logbook of serum, plasma, and fingerpick samples including:

10.2.1. Subject ID number

10.2.2. Date sample taken

10.2.3. Hb, HbA1c, and lipids

10.2.4. Make notes of any problems or anything out of the ordinary events, e.g., sample stood too long on bench, small samples, sample haemolysed, etc.

10.2.5. Missed Samples – mark with an "X' on the Serology tube lid and note on Inventory

10.2.6. The log should also note who the phlebotomist was, and who processed the blood samples.

10.2.7. This paper logbook system would continuously be updated, as new participant arrives on site, a new one would be generated.

10.2.8. File all paperwork away in folder, and keep two electronic records somewhere.

Appendix E: Standard Operating Procedure for Weight Height Waist and Hip Circumference

9. Scope

This standard operating procedure applies to the Vegan Health Research Programme members using the Stadiometer, Scale, and measuring tape in the Nutrition Research Unit at Massey University.

10. Objective

2.1. The objective is to describe the procedure of training the research member or the delegated person using the stadiometer, scale, and measuring tape prior to the start of research, and to describe the procedure and the operation and maintenance of the instruments during the research.

2.2. It is important that the research members or delegated persons who measure height, weight, and waist and hip circumference use the same procedure to ensure continuity and consistency in readings.

2.3. Height and weight are measured to determine the participants' body mass index (BMI), and waist and hip circumference to determine waist to hip ratio.

11. Definitions

3.1. Stadiometer: It is a device used to measure height. It consists of a head plate, a base plate, a rod and a digital display unit.

3.2. Inbody 230 scale: It is a device used to measure body composition, and for the purpose of this study to measure weight only. It has a display window which shows the weight. It is switched on and off by pressing the button on the bottom right hand corner of the display window.

3.3. Measuring tape: It is used to measure waist and hip circumference

3.4. Body Mass Index (BMI): It is defined as the individual's body weight in kilogram divided by the square of his or her height in meter. It is expressed as BMI and the unit is kg/m^2.

3.5. Waist to hip ratio: Waist-to-hip ratio looks at the proportion of fat stored on the body around the waist and hips. It is a simple but useful measure of fat distribution.

12. Responsibilities

4.1. The members of the research team are responsible for the following SOP.

4.2. The designated research member is responsible for ensuring that this SOP is followed.

13. Procedures

5.1. Instruments

5.1.1. Stadiometer: SECA 510

5.1.2. Scale: Inbody 230

5.1.3. Measuring tape: Lufkin measuring tape

5.2. Prior to research – Train observer

5.2.1. Train the observer how to measure height, weight and waist and hip circumference to the highest possible standard.

5.3. During research

5.3.1. Taking height measurements

5.3.1.1. Explain the procedure to the participant before starting to take the measurement

5.3.1.2. Ask the participant to remove their shoes

5.3.1.3. Raise the head plate to ensure that there is sufficient room for the participant to stand underneath it.

5.3.1.4. Ask the participant to stand with their feet flat on the centre of the base plate.

5.3.1.5. Ask the participant to put their feet together.

5.3.1.6. Ask the participant to put their heels against the wall.

5.3.1.7. Instruct the participant to have their back as straight as possible, preferably against the wall but not leaning on it.

5.3.1.8. Ensure that the participant is standing as tall as possible. Position the participant's head in the Frankfurt Plane position (the lower border of the left orbit and the upper margin of the external auditory meatus are horizontal).

5.3.1.9. Ensure that the participant keeps their eyes focused on a point straight.

5.3.1.10. Ask the participant to breathe in deeply.

5.3.1.11. Move the head plate so that it rests on the participant's head. If it is difficult for the investigator to determine whether the head plate is resting on the participant's head, the investigator can ask the participant to tell the investigator when the participant feels it touching their head by raising a hand.

5.3.1.12. Take the readings from the digital display unit and record the participant's height in centimetres and millimetres at the question "Height 1" in the Medical History Questionnaire. If a measurement falls between two millimetres, it should be recorded to the nearest even millimetre.

5.3.1.13. Ask the participant to step off the stadiometer.

5.3.1.14. Push the head plate high enough to avoid any member hitting their head against it.

5.3.1.15. Record the reading at question Height Reading 1 in tthe "Study day checklist"

5.3.1.16. Repeat the process and take the second measurement. Record the reading at the question Height Reading 2 in the "Study day checklist".

5.3.2. Taking weight measurements

5.3.2.1. Turn the Inbody machine on.

5.3.2.2. Ask the participant to remove shoes, heavy coats, cardigans, and any heavy objects such as keys or changes from their pockets.

5.3.2.3. Ask the participant to stand on the footplate of the machine with their feet on the silver plates.

5.3.2.4. Ask the participant to have their hands hanging loosely at their sides

5.3.2.5. Ask the participant to have their head facing forward and to look ahead. Be careful, it might be tempting for the participant to look down to read their

weight. Ensure the participant that you will tell their weight afterwards if they want to know.

5.3.2.6. Give a sufficient time for reading.

5.3.2.7. The Inbody machine displays the weight in kilograms and 100 grams units (0.1).

5.3.2.8. Record the reading at the question "Weight" in the "Study day checklist".

5.3.3. Taking waist circumference measurement

5.3.3.1. The participant should be standing with light/tight clothing.

5.3.3.2. Instruct the participant to stand with feet pointing forwards and approximately 25-30cm apart.

5.3.3.3. Their weight should be evenly distributed.

5.3.3.4. To position the tape, hold the casing of the tape in your right hand and with your left hand give the respondent the stub end of the tape (into their right hand) and ask them to pass it around their back and give it back to you.

5.3.3.5. Take hold of the stub with your right hand which then holds both the stub and the casing, leaving your left hand free to manipulate the tape at the correct level.

5.3.3.6. Use enough tension on the tape with the right hand to hold it where you position it.

5.3.3.7. Ask the respondent to put the tape at their waist level, i.e. the level at which it feels comfortable for them. When they have identified the level, use your left hand to ensure the tape is horizontal.

5.3.3.8. When you are happy with the tape position, reach underneath the casing with your left hand to take hold of the stub again and pull it across to your left into the cross-hand position, keeping enough tension on the tape to prevent it slipping out of position.

5.3.3.9. Move the tape sideways with both hands as needed to position the zero line nearer the respondent's side, rather than middle. When the tape is where you want it, remind the respondent to breathe normally. This is important as most won't without a reminder.

5.3.3.10. Apply gentle pressure on the tape – enough to ensure it is parallel, not indented and fairly firm. Take the reading to the nearest 0.1 cm, at the end of a normal expiration.

> Notes: When reading the tape, your eyes should be at the same level as the tape.
>
> When you have taken the reading, release the stub end of the tape and pull it gently around and off the respondent's trunk with your right hand. Take care that the stub end doesn't flick anywhere near your or the respondent's head.

5.3.3.11. Record the reading at the question Waist Circumference Reading 1 in the "Study day checklist".

5.3.3.12. Repeat this process for the second time and record the reading at the question Waist circumference Reading 2 in the "Study day checklist".

5.3.4. Taking hip circumference measurement

5.3.4.1. Following on from the waist measurement, ensure the participant remains in the same position, and breathing out gently.

5.3.4.2. With the tape measure the point yielding the maximum circumference. As before the tape should sit horizontally around the body, without a tilt.

5.3.4.3. Record the reading at the question Hip Circumference Reading 1 in the "Study day checklist".

5.3.4.4. Repeat this process for the second time and record the reading at the question Hip circumference Reading 2 in the "Study day checklist".

5.3.5. Calculations

5.3.5.1. Calculate the height, waist circumference, and hip circumference average by adding up readings 1 and 2 and dividing the result by two.

14. Care and maintenance

The equipment should be maintained on a regular basis to ensure that they are functioning well and they are hygiene.

6.1. Stadiometer

6.1.1. Clean the footplate with disinfectant wipe after each participant

6.1.2. Lubricate the stadiometer track at the end of the examination day

6.1.3. Check that the horizontal bar is firmly attached to the upright sliding section.

6.1.4. Check that the sliding section is functioning smoothly

6.1.5. If the sliding bar does not function smoothly, clean the bar with a damp cloth and lubricate the track.

6.2. Inbody 230

6.2.1. Clean the footplate with disinfectant wipe after each participant.

6.2.2. Clean the digital display with dry cotton. Ensure not to drip any fluid into the display housing

6.3. Measuring tape

6.3.1. Clean the measuring tape with disinfectant wipe after each participant.

6.3.2. Check the measuring tape every day before the visits to see if it is not torn, stretched or rubbed, and do not try to repair it because its scale indicators may become inaccurate. If any of these are observed use another measuring tape.

15. Precautions

7.1. Height measurement

7.1.1. Ensure that the same stadiometer has been used throughout the study period

7.1.2. Ensure to calibrate the stadiometer on the weekly basis

7.1.3. Ensure that participant removes their shoes to obtain a measurement that is as accurate as possible when taking height measurement

7.1.4. If the participant cannot stand straight upright with their back against the stadiometer and their heels against the wall then give priority to standing upright.
7.1.5. If the participant has a hair style which stands above the top of their head, or wears religious headwear, bring the head plate down until it touches the hair. To note, if a male person takes the measurement, it is strictly important to keep in mind that he should never touch the participant.
7.1.6. If the participant is tall, and it is difficult for the investigator to read the measurement, he/she can use a step

7.2. Weight measurement

7.2.1. Ensure that the batteries are relatively new and well charged
7.2.2. Ensure to calibrate the Inbody scale at the beginning of the study and on the daily basis. Although there is no need for frequent formal calibration, to ensure that the Inbody scale is functioning well it should be roughly calibrated.
7.2.3. Ensure that the same Inbody scale has been used throughout the study period
7.2.4. Ensure that participant removes their shoes, heavy jackets, cardigans, jewellery and any loose change and keys in the pockets when taking weight measurement
7.2.5. Ensure that the participant stands on the footplate of scale in the right posture. If the participant stands to one side, looks down or otherwise have their weight unevenly distributed, you might have inaccurate reading.
7.2.6. Ensure that the participant stands on the footplate of Inbody scale still and not moving excessively.

7.3. Waist and hip circumference measurement

7.3.1. Ensure that the measuring tape is not damaged
7.3.2. Ensure that the same measuring tape has been sued throughout the study period
7.3.3. Ensure for both waist and hip, the tape is snug around the body, but not pulled so tight that it is constricting, and at a level parallel to the floor.
7.3.4. Ensure that that the participant stands erect, with the body weight evenly distributed
7.3.5. Ensure that that the waist circumference is measured at minimal expiration
7.3.6. Ensure that the participant is relaxed and takes a few deep, natural breaths before the actual measurement is made, to minimize the inward pull of the abdominal contents.
7.3.7. Ensure that the participant has fasted overnight or in a fasted state because the amount of water, food or gas in the gastrointestinal tract affects the accuracy of the waist measurement.

16. References

8.1. World Health Organisation (2008). Waist Circumference and Waist-Hip Ratio - Report of a WHO Expert Consultation. WHO. Geneva, WHO.
8.2. Derived from [ARCHIVED CONTENT] (nationalarchives.gov.uk) on 29/01/2022

Appendix F: Question in Questionnaires Relevant to Iron Status

Have you ever been diagnosed with iron deficiency?

[ ] Yes

[ ] No

[ ] Unsure

If yes, please provide more details about your iron deficiency (self-diagnosed or diagnosed by a health care provider, date and treatment)……..

Do you get nose bleeds?

[ ] Yes

[ ] No

If yes, how often do you get a nose bleed?

_____ Times a month or _____ Times a year

If yes, how heavy are your nose bleeds?

[ ] Light

[ ] Medium

[ ] Heavy

Have you had any blood loss (other than periods or nose bleeds) such as wounds, regular scratches from contact sports, blood in stools, or urine in the past year?

[ ] Yes

[ ] No

If yes, please describe
below……..

Have you had any medical condition which has resulted in blood loss?

[ ] Yes

[ ] No

If yes, please describe and give approximate date
below……..

Do you donate whole blood (i.e. not plasma)?

[ ] Yes

[ ] No

If yes, when did you last donate blood?

Date _____ / _____ /_____

Day Month Year

If yes, how many times have you donated whole blood in the past year?

_____ (times in the past year)

Have you ever had iron infusion?

[ ] Yes

[ ] No

If yes, please provide details (reasons and date of infusion)

Have you had a blood transfusion in the last year?

[ ] Yes

[ ] No

If yes, please provide details (reason and date of transfusion)

Do you currently smoke?

[ ] Yes

[ ] No

If yes, how often do you smoke

[ ] Occasionally

[ ] A few times per week

[ ] Daily

If no, have you ever smoked?

[ ] Yes

[ ] No

If yes, how often did you use to smoke?

[ ] Occasionally

[ ] A few times per week

[ ] Daily

Are you currently taking any medication (excluding nutritional supplements)?

[ ] Yes

[ ] No

If yes, please state what medication you are taking and why?

Questions specific to women

1. Which of the following BEST describes your current Menstrual/Menopausal status?

[ ] Never menstruated
[ ] Still menstruating
[ ] Going through menopause
[ ] Postmenopausal
[ ] Other (Please explain)

2. Have you had a period in the last 3 months? (not including postmenopausal women)

[ ] Yes
[ ] No

3. How regular are your periods (21-34 days)?

[ ] Regular
[ ] Irregular

4. How many days do you usually have between periods? (for instance, counting from the first day of your last period to the day you expect your next period to start)
5. Do you know when your last period started?
6.

[ ] Yes
[ ] No

7. When did your last period start?

_____ / _____ /_____
Day Month Year

8. How many days does your period usually last?
9. Have you been pregnant within the last year?

[ ] Yes
[ ] No

10. If yes, did the pregnancy result in any significant blood loss that you are aware of? (Please comment below)
11. Are you on hormonal contraceptives?

[ ] Yes
[ ] No
[ ] Not applicable

12. If yes, please describe details (i.e. injection, IUD, implant, oral)

13. Do you currently take Hormone Replacement Therapy?

[ ] Yes
[ ] No
[ ] Not applicable

14. If yes, please provide more details (type of Hormone Replacement Therapy and for how long)

Appendix G: Food Diary Instructions

16.1

Health and Vegan Diet

16.2 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.

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) 1 cup So Good unsweetened almond milk 1 tsp Chelsea white sugar
Lunch – Meat Free Bacon Style Rashers sandwich and home-made fries	2 slices of wholegrain bread (Vogels) 2 slices Vegie Delights Meat Free Bacon Style Rashers 25g zenzo Dairy Free Vegan Cheddar Cheese Alternative 2 tsp Tablelands Dairy Free Buttery Spread ½ cup fries (home-made, deep fried in Pam's sunflower oil) ½ Tbs vegan aioli (Heinz Mayonnaise Vegan Aioli) Water 1 cup to drink
Dinner – Vegan lentils spaghetti bolognese	½ cup lentil sauce (see attached recipe) 1 cup spaghetti pasta (Homebrand)
Snacks	Tam & Luke Snack Ball Salted Caramel (2 balls, 28g)

	1 small banana 2 Salada crackers with 1 tsp peanut butter 20g Doritos Spicy Sweet Chili 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 2 large potatoes with skin (boiled)
Black bean and kumara burger	85g black bean and kumara burger (recipe provided) pan-fried in 2tsp olive oil 85g black bean and kumara burger (recipe provided) oven baked

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

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

- 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)

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 1 slice dairy free cheddar cheese (8.5 x 2.5 x 2mm) 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)
Example7: 55am	Sanitarium Weetbix	2 weetbix
" "	So good unsweetened almond milk	150ml
" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Home-made pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 meat free bacon style rashers (zenzo), 1 Tbsp chopped spring onion, 3 Tbsp vegan mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits	2 x Lotus Biscoff biscuits
6.00pm	Lasagne	½ cup cooked Sunfed Bull free beef meat alternative mince, 1 cup cooked Budget lasagne shaped pasta, ½ cup homemade (recipe attached) vegan bechamel sauce made with soy milk (So Good, regular), ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp Veesey grated pizza blend cheese
6.30pm	Vegan banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Crave dairy free salted caramel fudge frozen dessert	1/2cup (g) (125g)

Appendix H: Guide to Entering Food Diaries into FoodWorks

Note: a Vegan Codebook spreadsheet was also used to guide entering decision and can be made available upon request.

What terms should I consider when I search for a specific food?

Vegan, Plant-based, Vegetarian, Vegie (gives you more options!), Meat free, Chicken free

What factors should I consider when I want to make a substitution?

Ingredients – Are they comparable (particularly main ingredients)

Macronutrients – Are they comparable?

Micronutrients of interest (B vitamins, iron, Zn, sodium, calcium, phosphorus) – Are they comparable?

Look at both the packaging and grocery shops' website; the information provided may differ (e.g., Sunfed chicken)

Countdown website under ingredients and nutritional information section

Click on the photo and look for the nutrition information panel

What should I do if I don't find a substitution?

Enter the food as "Food" using override option –

- If you find a close option
- More nutrients would be available
- The preferred option

Enter the food as "Recipe" –

- if you don't find a close option to override but
- you have enough information in the ingredients part of label
- Main disadvantage- Based on assumptions

Enter the food as "Food" and don't use override option if you don't find a close option

- include the information from the nutrition information panel only
- Main disadvantage - Some nutrients and components would be missing

How can I put a recipe together when I have limited information?

Retention Factor (RF):Choose the raw ingredients and set the RF. If choosing the cooked ingredients don't assign a retention factor

Weight yield: Look at the main ingredients if the weight yield increases for one and decreases for the other one take the average

Serve weight: 100g

Then play around the amount of each ingredients to get the right macronutrient and micronutrients

Source of protein and protein quality

Source of protein:

Protein quality: PDCAAS (protein digestibility-corrected amino acid score) and DIAAS (Digestible indispensable amino acid score)

This has important implication for a vegan diet; Plant sources of protein have different and generally lower PDCAAS/DIAAS scores than animal sources (also affected by processing method). Example:

Milk: PDCAAS=100, DIAAS=100

Soy: PDCAAS=100, DIAAS=92

Pea: PDCAAS=91, DIAAS=66

Chickpea: PDCAAS=71, DIAAS=71

Fava/Faba: PDCAAS=67, DIAAS=?

Peanut: PDCAAS=55, DIAAS=47

If the plant protein is one of the main ingredients of the food (including desserts/snacks), and you don't find an appropriate alternative that is based on the same protein source – Make a "RECIPE" or include it as a "FOOD" – DON'T OVERRIDE

NOTE for assigning a name: include the protein source; e.g., chicken, 35% pea protein or chicken, 20% pea protein, 15% chickpea protein

Vegan Desserts and Snacks

Consumed occasionally – OVERRIDE

A NOTE about overriding:

Look at those ingredients that you think that they have been substituted by plant based alternatives.

Compare the nutritional profile of those ingredients and their alternatives.

Find those nutrients that are substantially different and then make adjustments

Milk (calcium, protein quality, saturated fat, cholesterol, iodine, etc.)

Egg (cholesterol, protein quality)

Butter - Type of fat/oil (saturated fat, trans fat, cholesterol, mono and polyunsaturated fatty acids)

NOTE: if one of these ingredients is the main ingredient, then you need to make a "RECIPE" or include it as a "FOOD" and DON'T OVERRIDE

E.g., milk-based puddings, cakes that contain a large amount of eggs or/and butter (sponge cake)

Fortification/added nutrients

Label

NIP and ingredients (some don't mention that the product is fortified with a nutrient)

OVERRIDE – using not fortified alternatives and make adjustment regarding the added nutrients

Appendix I: Supplementary Results

The below table shows the number of female participants meeting and not meeting the recommended dietary requirements (RDI) and estimated average requirements (EAR) for iron based on menstrual status. The RDI and EAR relevant to the different menstrual statuses are in bold.

Table A. Female Participants at Different Menstrual Stages and Numbers Meeting Recommended Dietary Intake and Estimated Average Requirements Targets

	RDI for menstruating females (19-50 years) (n (%))		RDI for non-menstruating females (50-70+ years) /EAR for menstruating females (19-50 years) (n (%))		EAR for menstruating females (19-50 years) (n (%))	
	<18mg/day	≥18mg/day	<8mg/day	≥8mg/day	<5mg/day	≥5mg/day
Never menstruated	0 (0.0)	1 (0.8)	**0 (0.0)**	**1 (0.8)**	0 (0.0)	1 (0.8)
Menstruating	**60 (46.0)**	**36 (27.7)**	**3 (2.3)**	**93 (71.5)**	**1 (0.8)**	**95 (73.1)**
Menopause	**4 (3.1)**	**6 (4.6)**	**0 (0.0)**	**10 (7.7)**	**0 (0.0)**	**10 (7.7)**
Postmenopausal	12 (9.2)	11 (8.5)	**0 (0.0)**	**23 (17.7)**	0 (0.0)	23 (17.7)

The following table displays the number of menstruating females in each serum ferritin category.

Table B. Iron Status of Menstruating Women

Serum Ferritin Status	Menstruating Females (n (%))
SF <20µg/L (n (%))	36 (33.6)
SF 20-30µg/L (n (%))	34 (31.8)
SF ≥30µg/L (n (%))	37 (34.6)